THE ROLE OF MYCORRHIZAS IN DROUGHT RESISTANCE OF SITKA SPRUCE SEEDLINGS

by

Tarja Helena Lehto

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CONTENTS

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ABSTRACT

The contribution of mycorrhizas to the drought resistance of Sitka spruce *(Picea sitchensis* (Bong.) Carr.) seedlings was studied, with regard to their short-term physiological effects, and longer term effects on plant growth and nutrition during drought.

Seedlings either inoculated with *Paxillus involutus* (Batsch) Fr, or nonmycorrhizal were subjected to a single drying and rewatering cycle in a controlled environment. When plants were grown in vermiculite-peat, mycorrhizal plants had much larger P and K concentrations, they were larger, and their net photosynthetic rates were higher than for nonmycorrhizal plants. Water potentials of well-watered mycorrhizal plants were higher than those of nonmycorrhizal plants, and during a drought treatment, the larger mycorrhizal plants dried their substrate out sooner, yet their stomatal conductances, net assimilation rates, and shoot water potentials were similar to droughted nonmycorrhizal plants.

When the experiment was repeated on mycorrhizal and nonmycorrhizal plants of comparable size and N, P, and K concentrations, the water potentials of mycorrhizal plants were slightly lower than those of nonmycorrhizal plants at the same soil water status, and their stomatal conductances and photosynthetic rates were not higher than those of nonmycorrhizal plants. It was concluded that the mycorrhizal effects in the first experiment were caused rather by better nutrition and larger root and mycelial systems than by intrinsically larger soil-to-plant conductance in mycorrhizal plants.

Seedlings inoculated with *P. involutus* or *Thelephora terrestris* (Ehrh.) Fr., or nonmycorrhizal, were subjected to moderate drying and rewatering cycles to find out if the mycorrhizal structure would induce more sustained growth of shoots and root systems and nutrient uptake. The root / shoot ratios of nonmycorrhizal plants were increased in dry conditions more than those of mycorrhizal, but the differences between the two inoculation treatments tended to be larger than differences between mycorrhizal and nonmycorrhizal plants. *T. terrestris* inoculated plants were generally least affected by drought; their root tip numbers did not decrease as in other treatments, and their nutrient contents were larger.

Effects of drought on mycorrhizal formation of *P. involutus*, *T. terrestris*, *Hebeloma crustuliniforme* (Bull.: St Amans) Quél. and *Laccaria proxima* (Boud.) Pat. were studied during well-watered, moderate, and severe drying cycles. *T. terrestris* formed abundant mycorrhizas in all watering treatments, *P. involutus* showed some preference to the moderately droughted regime, and *L. proxima* and *H. crustuliniforme* formed most mycorrhizas in the well-watered treatment. These differences were not reflected in the growth of plants, but the shoot nutrient status was related to the mycorrhizal proportion. The differences between the fungi were concluded to be important in terms of their mycorrhizal formation and effects on root tip initiation in dry conditions, which will enable plants to take up nutrients during drought.

CHAPTER 1
INTRODUCTION

1.1 Background

Coniferous seedlings produced in forest tree nurseries are often poorly mycorrhizal, particularly those grown in containers. There are several reasons for this: seedlings are grown in high fertilizer levels inhibiting mycorrhizal formation, in substrates with low inoculum potentials such as peat, under a polythene cover which prevents or delays spread of airborne spores (Molina 1980). Bare-rooted transplants are usually mycorrhizal in northern temperate and boreal areas, but there is concern as to whether the species and strains of mycorrhizal fungi common in nurseries are adaptable to the field, as the conditions at planting sites are quite different from those in nurseries. Seedlings are normally planted in the spring, when the soil is cold and often dry, but the evaporative demand of the air may be high. the interaction of these environmental factors and the fact that the carbohydrate reserves of outplanted seedlings may have been depleted during storage, and the root systems may be deficient or damaged, leads to a condition known as planting check. Water stress is one of the most important components in planting check, and may be the major reason for low survival rates of seedlings or loss of growth (e.g. Hallman *et al.* 1978, Grossnickle 1988a,b). Hence, if it can be demonstrated that lack of mycorrhizal infection or infection with an unsuitable fungus exacerbates the water stress experienced by seedlings, this should be taken into account in practical inoculation programmes.

1.2 Hypotheses on the role of mycorrhizas in plant water relations

The role of mycorrhizas in the water relations of plants is not well understood, even though their importance in nutrient uptake has long been recognized. It has been a common notion that most of the water uptake by ectomycorrhizal plants could be accounted for by the older parts of the root systems which have undergone secondary growth and suberization (Kramer & Bullock 1966), and perhaps for this reason nutrient uptake was considered the single most

important function of ectomycorrhizas. It was not until the 1970's that some of the pioneering experiments were done, actually showing with tritiated water that water uptake rates by extraradical strands of ectomycorrhizal fungi can be of the same order of magnitude as those of roots (Duddridge *et al.* 1980).

The most likely way in which ectomycorrhizas may affect plant water relations is to increase water uptake, which would result in larger stomatal conductance and hence increased carbon assimilation in dry conditions (Reid 1979). Increased water uptake by mycorrhizas as opposed to nonmycorrhizal roots may be effected by an increase in absorbing surface area, or external mycelium providing a low resistance pathway for water flow through the soil (Reid 1979). Also, mycorrhizal roots and their external mycelium may be less inclined to shrink and thereby form gaps between soil surfaces and absorbing surfaces (Dosskey & Ballard 1980). In the context of vesicular-arbuscular mycorrhizas, it is increasingly being appreciated that improved plant nutrition due to mycorrhizal structure can lead to increased conductance to water in the soil-fungus-plant-atmosphere continuum (Safir *et al.* 1972, Fitter 1988), but there is also evidence of increased water uptake by hyphae.

Read & Boyd (1986) suggested that benefits of infection are likely to arise particularly in stress conditions, in analogue to mycorrhizal benefits in mineral nutrition. Mycorrhizas could be of benefit through their capacity to provide the minimum requirements for survival during stress rather than through their ability to sustain high flow rates. In view of this, the advantage from mycorrhizal structure may not be manifested unless there is a prolonged or repeated drought. In the longer term the ability of the fungus to grow and form mycorrhizas, as well as to stimulate root growth, may be important, as this is a prerequisite for sustained water uptake and nutrient uptake from dry soil.

The initial hypothesis tested in this thesis is that mycorrhizas increase short-term drought resistance of Sitka spruce independently of nutrient effects. The second hypothesis is that mycorrhizas increase plant resistance to repeated droughting by enhancing root growth and nutrient uptake, and that this effect is related to the ability of the particular fungal species to form mycorrhizas in dry soil.

1.3 Objectives and thesis plan

The objectives of this thesis are:

(1) To compare ectomycorrhizal and nonmycorrhizal Sitka spruce seedlings in terms of drought resistance, using gas exchange and total water potential to assess short-term effects of drought.

(2) To assess effects of repeated drying and rewatering on root and shoot growth, mycorrhizal formation and nutrient uptake in nonmycorrhizal and different mycorrhizal plants.

In the first part of the thesis, the main emphasis is on the effects of a drought treatment on previously well-watered, mycorrhizal and nonmycorrhizal Sitka spruce seedlings. After experiments with uncontrolled nutrition (Chapter 4), and developing a technique for growing comparable mycorrhizal and nonmycorrhizal plants (Appendix B), the same type of experiments are reported on plants with little mycorrhizal effect on growth and nutrition (Chapter 5). In Chapter 6, an experiment on tissue water relations of differentially fed plants is described, to elucidate some nutrient effects that could explain the differences between results from the first experiments.

In the latter part of the thesis, the emphasis is on effects of repeated drying cycles on different mycorrhizal and nonmycorrhizal plants. Chapter 7 is concerned with effects of several moderate drying cycles on the allocation of growth between roots and shoots and at different depths of the root systems, as well as nutrient accumulation in mycorrhizal and nonmycorrhizal seedlings. In the last experiment reported (Chapter 8), the drying cycles are imposed on plants from the time of inoculation, to study their effects on the mycorrhizal formation of four fungi, and whether this can be related to the effects of droughting on the growth, allocation of growth, and nutrient accumulation of the plants.

CHAPTER 2
LITERATURE REVIEW

2.1 Whole-plant responses to water deficits

2.1.1 Water transport and photosynthesis

In the following literature review, effects of water stress on plants are first discussed, with emphasis on those aspects on which ectomycorrhizal infection is thought to have an effect, as well as currently controversial topics. As mycorrhizas are known to have a very significant role in plant nutrition, the interactions of nutrient and water relations are discussed in a separate section before moving to experimental work on ectomycorrhizas and plant water relations. To provide background information, some general aspects on ectomycorrhizas, and drought resistance of vesicular-arbuscular mycorrhizal plants are discussed briefly. A general review of plant water relations is found in Kramer (1983). The movement of water in the soil-plant-atmosphere continuum has been recently discussed by Passioura (1982, 1988), Boyer (1985). Tissue water relations have been reviewed by Tyree & Jarvis (1982), and osmotic adjustment by Morgan (1984), especially in conifers by Abrams (1988). The responses of stomata to environmental factors, and the importance of this to the control of water loss and CO_2 assimilation have been treated comprehensively by Boyer (1975), Jarvis (1980), Schulze & Hall (1982), and Schulze (1986), and the contribution of mycorrhizas to the drought resistance of plants by Reid (1979) and Read & Boyd (1986).

Contemporary models of water movement in the soil-plant-atmosphere continuum are based on thermodynamic concepts: a water potential gradient is seen as the driving force for water flow, which is affected by resistances in the different parts of the pathway. Use of this approach has made it possible to express the water status of the plant and the soil in the same units, and to treat water flow in the soil-plant system as a whole (Kramer 1988). However, the applicability of the approach, especially the use of water potential, has been questioned for several reasons. Plants may have means for more active control of shoot water status by stomatal closure than earlier believed (this is

discussed below in the context of stomatal behaviour). Moreover, the water flow in the soil and plant is governed by a hydrostatic pressure gradient (turgor in plants) rather than water potential with the exception of water flow across membranes, and turgor is a rather unstable variable (Sinclair & Ludlow 1985). As there is no evidence of any universal relationships between water potential and water stress -induced metabolic changes, although many of these changes are related to shoot or leaf relative water content (RWC), Sinclair & Ludlow (1985) proposed the use of RWC rather than water potential as a measure of plant water status. Another advantage of their 'water balance' approach is that RWC can be related to changes in turgor pressure, and as it is more stable than water potential or its components, it is likely to be an expression of more meaningful changes in plant water status. A major disadvantage of the RWC is uncertainty in its measurement.

Nevertheless, Boyer (1989) pointed out that the use of water potential and its components is still essential for understanding water transport and cell enlargement, and as a means of comparing experiments.

The pathway for liquid water flow in soil and in the plant can be represented as a network of resistances. The major resistances on the pathway are thought to be in the soil, at the soil-root interface, and within the plant. The soil resistance is small relative to the other components, except in sandy soils with very low rooting densities (Sands & Theodorou 1978). More recently, a contact or interfacial resistance has been postulated to explain discrepancies in models of uptake of water from soil (Passioura 1988). This is a resistance at the soil-root interface caused by incomplete contact of wet soil surfaces and absorbing surfaces.

The relative importance of the resistances within the plant varies from species to species. In tall trees, there is considerable resistance in the trunk, and this has been found to be larger per unit length of trunk for Sitka spruce than for pines and angiosperms (Hellkvist *et al.* 1974). However, in small seedlings the root resistance, or the resistance between the soil-root interface and the xylem, tends to be the largest component (Passioura 1988). Water has to cross a membrane in the root either at the endodermis, if the major pathway in the cortex is in the apoplast, or before that, if the major pathway is in the symplast (Weatherley 1982), which imposes a large resistance to flow.

The ability of plants to regulate transpiration rates by opening and closing their stomata is well established; the stomatal conductance of Sitka spruce is linearly correlated to saturation deficit over a wide range of deficits (Jarvis 1980). Stomatal closure in response to low leaf water potential is well-known, too (Jarvis 1980). However, in field conditions needle water potentials rarely fall so low as to cause stomatal closure in Sitka spruce trees, because there is a threshold water potential for closure which is outside the range of potentials reached due to normal diurnal changes in transpiration rates (Jarvis 1980). Moreover, soil water deficits likely to cause low leaf water potentials are usually coupled with low atmospheric humidity, which is the primary factor causing daytime stomatal closure in Sitka spruce canopies in the field (Watts *et al.* 1976), and therefore stomatal closure tends to prevent leaf water potentials from falling to the threshold value which would affect stomata in its own right. Furthermore, osmotic adjustment tends to decrease the threshold water potential for stomatal closure. In situations when stomata have closed in response to leaf water deficits, there is often a time lag in their reopening after the water potential has recovered, suggesting an additional role of some other regulator (Ackerson 1982, Buxton *et al.* 1985).

Another reason why stomatal closure due to low leaf water potentials may be rare in seedlings, is a direct response of stomata to increasing soil water deficit (as well as other disturbances in the rhizosphere such as mechanical damage; Coutts 1980, 1982b). Coutts (1981) found that stomatal opening of Sitka spruce seedlings was more closely coupled to soil water deficits than to leaf water potential; stomatal conductance of potted seedlings was reduced during drought when the daytime leaf water potential varied between −1.0 and −1.3 MPa, as opposed to results on detached shoots showing that stomata of Sitka spruce trees were insensitive to leaf water potentials above −1.4 MPa (Beadle *et al.* 1979). The coupling of stomatal opening to soil conditions may be caused by roots sensing changes in soil conditions and sending a chemical 'message' to shoots. Evidence to support this concept is accumulating from experiments showing stomatal closure in plants with a high leaf water status but part (Blackman & Davies 1985) or all of their root systems (Gollan *et al.* 1986) exposed to drying soil. This effect was initially thought to be mediated by a decrease in synthesis of cytokinins in roots and transport to shoots (Davies *et al.* 1986), but more recent studies suggest an increase in abscisic acid produced in roots in dry conditions (Zhang & Davies 1987, 1989).

In controlled environment experiments on potted coniferous seedlings, a common response pattern to drying soil is that plants use a large part of the soil moisture, keeping their leaf water potentials nearly constant (Watts & Neilson 1978, Havranek & Benecke 1978). At some threshold value of soil moisture content the plant water potential decreases rapidly; this corresponded to the decrease in soil water potential in experiments by Havranek & Benecke (1978) on *Larix decidua* Mill., *Picea abies* (L.) Karst. and *Pinus cembra* L.

Photosynthesis is thought to be affected by water deficits in two different ways, which result from changes in stomatal conductance to CO_2, and in the photosynthetic apparatus of the leaves. With increasing water deficit, stomatal conductance decreases, and with it, the CO_2 supply for photosynthesis. However, Farquhar and Sharkey (1982) argued that the role of stomatal opening has been greatly exaggerated in many studies into stress effects on photosynthesis, as stomatal closure may diminish transpiration rates with decreasing water supply without diminishing the photosynthetic rate. On the contrary, Downton *et al.* (1988) considered many previous reports on nonstomatal inhibition of photosynthesis during stress, based on calculations of intercellular CO_2 levels, as erroneous, and being caused by groups of stomata closing rather than the photosynthetic apparatus having been affected by water stress. These examples from the literature highlight the controversy concerning the mechanisms of the decrease in photosynthesis during drought. Jarvis & Sandford (1986) considered much of this discussion fruitless, as it has often been based on inadequate assessments of the sites of action of the stress. Nevertheless, the net assimilation rate as such remains a valid indicator of the strain in the plant, especially for comparative purposes.

2.1.2 Growth and allocation of resources

The expansion of cells is directly affected by water deficit, as it is driven by the difference between the actual turgor pressure and the threshold yield turgor of cell walls, and affected by the water potential gradient in the plant (Passioura 1982). A similar relationship has been observed between the turgor of fungal hyphae and their growth, even though there is much less information on the water relations of fungi than those of higher plants (Eamus & Jennings 1986). The growth of hyphae is thought to be driven by solute accumulation in the fungal tips and hence increased water uptake and increased turgor (Eamus &

Jennings 1986).

Reduction in leaf expansion can be seen as an advantage to plants as it both reduces the growth of transpiring surface and hence water loss, and releases resources for root growth, enabling water uptake from previously unexploited soil volumes, if such are available. Enhanced root growth relative to shoot growth may involve increased hydrolysis of root starch as in Douglas fir *(Pseudotsuga menziesii* (Mirb.) Franco; Marshall 1986) or also increased translocation of carbohydrates to roots as in lodgepole pine *(Pinus contorta* Dougl.; Reid & Mexal 1977). Sometimes moderate drought causes an increase in root growth in absolute terms, not only relative to shoot growth. This has been observed in red pine *(Pinus resinosa* Ait.; Becker *et al.* 1987), maize *(Zea mays;* Zhang & Davies 1989) and flax *(Linum usitatissimum* L.; Menoux 1979). When the outplanted red pine seedlings were exposed to more severe drought, their root length and mycorrhizal numbers per root length were reduced relative to watered controls (Becker *et al.* 1987). In Norway spruce *(Picea abies)* drought suppressed the growth of parent roots but enhanced mycorrhizal growth, leading to higher branching density both in a field experiment and a pot experiment (Feil *et al.* 1988)

However, in the longer term root growth is reduced more markedly, as observed in *Pinus taeda* L. and *P. strobus* L. subjected to several drying and rewatering cycles (Kaufmann 1968). Moreover, increases in root / shoot ratios in response to drought have not always been found in experiments, possibly because of severity of drought treatments. Jarvis & Jarvis (1963) found decreased root / shoot ratios and decreased shoot growth in *Pinus sylvestris* L., *Picea abies* and *Betula pendula* Roth. (*B. verrucosa* Ehrh.) in mean soil water potentials of about -0.17 and -0.33 MPa. They did not consider this treatment severe droughting, consistently with the earlier concept that there are no considerable losses in plant growth before plants are exposed to soil water potentials near the wilting point, -1.5 MPa. However, the growth of first year *Pinus sylvestris* shoots was reduced when they were grown in as high soil water potentials as -0.03 MPa (Sands & Rutter 1959).

Root growth of Sitka spruce appears to be more sensitive to dry conditions than that of many other species (Coutts 1982a). When root systems of Sitka spruce seedlings were split between soils of unequal matric potentials, shoot growth was reduced only by a small amount if part of the root system was in

moist soil, but root growth was suppressed in the dry compartment whether the other half was in dry or moist soil (Coutts 1982a). This was compensated for by larger root dry weight increment in the wet compartment, and the total weight of root systems was not different in the watered and unequally watered plants. However, roots did grow in the dry soil, and it was suggested that the dieback of roots approximately balanced the new growth.

2.2 Plant nutrition and water relations

2.2.1 Effects of nutrient status on water relations

Studies of the effects of mineral nutrients on plant water relations are somewhat conflicting in their results, but there is some similarity in the effects of nitrogen, phosphorus, and potassium deficiencies, probably because plants have relatively few ways of responding to different environments. Especially reduced stomatal conductance and reduced root hydraulic conductance have been reported as a result of deficiency of each of these major nutrients.

Nitrogen deficiency has been reported to induce structural changes which are characteristic of drought resistant plants: smaller leaf areas, smaller epidermal cells, thicker and more rigid cell walls (Shimshi 1970, Radin & Parker 1979a, Morgan 1986). The structural changes were associated with lower osmotic pressure and lower tissue elasticity (smaller change in turgor for a given change in relative water content) in N deficient wheat plants *(Triticum aestivum* L.; Morgan 1986). During a drought treatment, the deficient plants had higher relative water contents. However, the water use efficiency was always higher in the high-N plants despite the other traits which may be interpreted as drought resistance in the low-N plants. Radin & Parker (1979a) also found lower tissue elasticity and slightly lower osmotic potentials in N deficient cotton plants *(Gossypium hirsutum* L.), but when these were exposed to dry conditions they did not exhibit more extensive osmotic adjustment than high-N plants (Radin & Parker 1979b). Therefore the lower tissue elasticity may not be of particular advantage during drought, since low elasticity without concomitant osmotic adjustment would lead to considerable turgor loss with small decreases in relative water content (Morgan 1986).

The stomata of N deficient wheat plants were less sensitive to decreasing leaf water potential (Morgan 1986), but the N deficient cotton plants were more sensitive (Radin & Parker 1979b). A similar increase in stomatal sensitivity to water stress was found in N deficient tea plants (*Camellia sinensis* L.) by Nagarajah (1981), and Shimshi (1970) suggested that N deficiency impaired the ability of bean plants (*Phaseolus vulgaris* L.) to adapt to water deficits by adjusting stomatal closure and sap-solute concentration; the deficient plants had lower transpiration rates than controls in watered soil, but higher in dry conditions. Radin & Parker (1979b) argued that the stomatal sensitivity to leaf water status was a characteristic independent of osmotic adjustment or other means of turgor regulation. They suggested that the increased sensitivity of stomata in N deficient plants was caused by both increased accumulation of abscisic acid (ABA), and increased sensitivity of stomata to ABA (Radin & Ackerson 1981, Radin *et al.* 1982). In their experiments, the soil water status was not recorded.

Little work has been done on conifers regarding nutritional effects on water relations. In a field experiment, Brix (1972) found higher water potentials in N-fertilized Douglas fir even though fertilization also decreased soil water potentials. Therefore he concluded that the fertilization effect was on increased water uptake and possibly improved water use efficiency.

Phosphorus deficiency has been found to decrease stomatal conductances in watered cotton (Ackerson 1985, Radin & Eidenbock 1984) and *Beta vulgaris* L. (Terry & Ulrich 1973a), and transpiration rates in watered *Arctium minus* Bernh. (Atkinson & Davison 1972). However, the transpiration rates of droughted P-deficient *Arctium* plants were higher than those of droughted plants with adequate P. Similarly, transpiration of tea plants was decreased by P deficiency as a combined result of lower stomatal density and aperture, but it declined more slowly during drought (Nagarajah & Ratnasuriya 1978). Without indicating the plant or soil water status, Atkinson & Davison attributed this apparent lack of sensitivity to impaired stomatal functioning, but Nagarajah & Ratnasuriya showed that in the P deficient tea plants it was a result of reduced depletion of water from the substrate despite the similar size of plants in the high-P and low-P treatments, and the sensitivity of stomata to water stress was not affected. However, the recovery from water stress of deficient tea plants was slower. Syvertsen & Graham (1985) also observed lower transpiration rates in *Citrus* rootstocks with low N and P levels, and Radin &

Eidenbock (1984) reported lower leaf water potentials associated with the lower transpiration rates in P deficient cotton plants than plants with adequate P. In contrast to other investigators, Radin (1984) found that the stomata of P deficient cotton plants were more sensitive to decreasing leaf water potentials, and as there was also an increased stomatal response to abscisic acid in deficient plants, he suggested that this was the reason for the increase in stomatal sensitivity, as the turgor pressure was decreased only slightly in the deficient plants.

Potassium deficiency has been reported to decrease transpiration or stomatal conductance in watered *Medicago sativa* L. (Cooper *et al.* 1967), *Zea mays* L. (Peaslee & Moss 1968, Koch & Estes 1975), *Camellia sinensis* (Nagarajah 1979), *Beta vulgaris* (Graham & Ulrich 1972), *Prunus domestica* L. (Evans *et al.* 1977 and *Pinus sylvestris* (Christersson 1976). However, sometimes the opposite has been found, possibly due to higher initial K status of the plants: in *Pinus sylvestris* (Christersson 1973), *Triticum aestivum* and *Pisum sativum* L. (Brag 1972), and *Picea sitchensis* (Bradbury & Malcolm 1977). The potassium effect is caused rather by stomatal opening than by different stomatal density (Koch & Estes 1975, Evans *et al.* 1977), and therefore it may be directly related to the role of K^+ in stomatal function (Nátr 1975, Graham & Ulrich 1972).

The lower transpiration rates in K deficient plants have been associated with higher leaf water potentials (Evans *et al.* 1977, Nagarajah & Ratnasuriya 1978), but earlier closure of stomata in response to dry soil (Nagarajah & Ratnasuriya 1978) and decreased CO_2 uptake (Koch & Estes 1973, Peaslee & Moss 1968). The higher transpiration rates in low-K Sitka spruce in turn were associated with low water use efficiency, and the sensitivity of stomata to leaf-air vapour pressure deficit was increased as a result of P and K fertilization (Bradbury & Malcolm 1977).

Lowered root hydraulic conductances have been found as a result of a deficiency of N, P and K (Radin & Boyer 1982, Syvertsen & Graham 1985, Radin & Eidenbock 1984, Coleman *et al.* 1987, Graham & Ulrich 1972), and this has been seen as one of the primary ways in which nutrition can affect plant water relations and growth. N deficient sunflower *(Helianthus annuus* L.) plants with low root conductances had low daytime leaf water potentials, and they were not able to maintain a high turgor. As the threshold turgor for growth and the plastic extensibility of the cells was not affected by N nutrition, the decreased

extension growth was interpreted as a result of the decreased root conductance (Radin & Boyer 1982). In P deficient cotton plants, water potentials, transpiration rates and root conductances were lower than those of plants with adequate P before any difference in leaf expansion was observed, and therefore Radin & Eidenbock (1984) suggested that the decreased leaf expansion due to P deficiency was caused by decreased root conductance. Similarly, Syvertsen & Graham (1985) found that leaf N and P status in different *Citrus* rootstocks was related to their root conductance, and they suggested that the higher stomatal conductances and hence photosynthetic rates in the plants which were able to extract more nutrients from the soil were due to different root conductances.

Clearly, nutrient and water interactions have an important yet not conclusively studied role in the physiology of plants. The conflicting results are at least partly due to nutritional effects depending on many factors such as plant species, age, water stress history, and the levels of nutrients involved (Shimshi 1970, Christersson 1976). Despite this, the internal concentrations of the nutrient in question have not always even been reported in publications. Plant size and growth rate is a further complicating factor (Morgan 1986); as these changes occur simultaneously with physiological changes such as those in stomatal conductance, root conductance or tissue elasticity, the conclusion often is that cause and effect relationships cannot be drawn from correlations (Syvertsen & Graham 1985).

2.2.2 Nutrient uptake during drought

Nutrient uptake by plants during drought has been reviewed by Viets (1972). Soil water affects nutrient availability through its effects on concentrations of nutrients, on rates of diffusion and mass flow, and on root absorption (Viets 1972). Nutrient uptake has been conclusively shown to be reduced in dry soil, e.g. Olsen *et al.* (1961) showed a linear relationship between phosphorus uptake of corn plants *(Zea mays)* and the soil moisture content. Dunham & Nye (1976) discussed the relative importance of soil factors, or the reduction in transport to the root surface, and the plant factors, which they named 'nutrient absorbing power', in the reduction of P and K uptake from dry soil. They concluded that the reduction in K uptake was caused primarily by a reduction in transport, whereas the reduction in root absorbing power was more important for P.

Because of the clearly adverse effects of drought on nutrient uptake, the reduced growth of plants in dry soils has sometimes been partly attributed to reduced nutrient uptake (Mengel & Kirkby 1982).

2.3 Ectomycorrhizas and plant water relations

2.3.1 The role of ectomycorrhizas in plant carbon and mineral nutrition

The importance of ectomycorrhizas in plant nutrition is well established; since the early studies by Melin (1927) and Björkman (1942) a substantial amount of evidence has accumulated to show that particularly nitrogen and phosphorus uptake, but also the uptake of other mineral elements is enhanced by mycorrhizal structure as opposed to nonmycorrhizal roots (Bowen 1973, Harley & Smith 1983). In spite of this, there may not always be much difference in the nutrient absorbing power of mycorrhizal and nonmycorrhizal roots. Reid & Bowen (1979) reported very similar phosphate absorbing rates for young nonmycorrhizal and mycorrhizal roots of coniferous seedlings per unit surface area, even though the phosphate absorption was larger in mycorrhizal roots per unit fresh weight. Also, Ingestad *et al.* (1986) were unable to show an inherently greater nutrient uptake capacity of ectomycorrhizal root systems in liquid culture as opposed to nonmycorrhizal, and they concluded that the mycorrhizal enhancement of nutrient uptake is caused by indirect factors. Some of the other mechanisms are now understood. The high absorbing power of mycorrhizas is more sustained, whereas that of nonmycorrhizal roots in any one place in soil decreases rapidly with time (Bowen 1973), and the mycorrhizal mycelium forms a functional extension of the relatively sparse root systems of ectomycorrhizal host species (Bowen 1973, Duddridge *et al.* 1980). Mycorrhizas are also able to break down and take up organic compounds; ectomycorrhizal fungi produce phytases (Gianinazzi-Pearson & Gianinazzi 1986); they can enhance the production of acid phosphatases (Williamson & Alexander 1975); they are able to produce proteolytic enzymes (Read *et al.* 1989) and take up amino acids which are inaccessible to host plant roots (Lundeberg 1970, Alexander 1973, 1983). There is also a possibility that mycorrhizal fungi can dissolve inorganic phosphates in some soils by acidifying their environment (Gianinazzi-Pearson & Gianinazzi 1986).

The growth and metabolism of the mycorrhizal fungus induces a sink of carbon to the host plant; the fungus uses current photosynthate from the host (Harley & Smith 1983, Söderström & Read 1987), and many times more photosynthate may be translocated to ectomycorrhizal than nonmycorrhizal roots of the same plants (Bevege *et al.* 1975). Therefore the photosynthetic rate of the host plant may be expected to increase as a result from infection, according to the concept that the sink size is a regulator of photosynthetic rates. This would be an effect parallel to the mycorrhizal effects mediated by mineral nutrition, and hence rather difficult to study experimentally. Much of the experimental work on the carbon balance of ectomycorrhizal and nonmycorrhizal plants has been confounded by differences in the mineral nutrition of the plants.

The photosynthetic rates of mycorrhizal *(Suillus granulatus* (L.: Fr.) Kuntze and *Pisolithus tinctorius* (Pers.) Coker and Couch) *Pinus contorta* and *P. taeda* seedlings were considerably higher than those of nonmycorrhizal (Ekwebelam & Reid 1983, Reid *et al.* 1983), and allocation of photoassimilated ^{14}C in the root systems as well as root respiration were higher, when the mycorrhizal seedlings had higher P concentrations (Reid *et al.* 1983). In contrast, Ahrens & Reid (1973) did not find differences in quantities of soluble carbohydrates or their distribution between roots and shoots of mycorrhizal *(Cenococcum geophilum* Fr., *Thelephora terrestris* (Ehrh.) Fr. and *Rhizopogon vinicolor* A.H.Smith) and nonmycorrhizal *Pinus contorta* seedlings after exposure to $^{14}CO_2$, when the shoot and root dry weights were similar in the treatments. Moreover, when similar conditions of mineral nutrition were achieved, there was no difference in the assimilation rates of mycorrhizal *(Paxillus involutus* (Batsch) Fr.) and nonmycorrhizal Sitka spruce seedlings (Wilson, J., personal communication). Nylund & Unestam (1987) and Nylund & Wallander (1989) did find an increased photosynthetic rate as a result of infection of 'perfectly nourished' Scots pine with *Laccaria* and *Hebeloma* sp., but this was concomitant with a comparative decrease in their relative growth rates. Similarly, Ingestad *et al.* (1986) found lower relative growth rates in *Suillus bovinus* (L. ex Fr.) O.Kuntze mycorrhizal Scots pine than nonmycorrhizal seedlings of the same nitrogen status. Hence the photosynthetic rates were not increased enough to cover the sink in the fungus, when plants were supplied with soluble nutrients.

The conclusion that mycorrhizal infection may not increase growth unless mycorrhizas can substantially increase nutrient uptake, has also been reached

in experiments with containerized coniferous seedlings (Molina 1982, Shaw *et al.* 1982, Molina & Chamard 1983). However, in natural environments the carbon sink in the fungus may be compensated for by the lower maintenance respiration of mycorrhizal root systems (Marshall & Perry 1987), the relative longevity of functional mycorrhizas (Bowen 1973), and increased carbon assimilation due to improved nutrition.

The particular importance of understanding the carbohydrate physiology of mycorrhizal associations in context with their water relations is that, while water moves from the fungus to the plant as a result of transpirational pull, there is still a need for the fungus to obtain carbohydrates from the host plant to maintain hyphal growth. In mycelial strands, which take up water and transport it to the host plant along 'vessel' hyphae (Section 2.3.4.), this must occur against a mass flow of water. There are two possible ways of explaining the fact that hyphal growth does occur: spatial differentiation of the transport function, as in plants, and temporal differentiation, which would involve water uptake during the day, and carbohydrate translocation during the night (Read & Boyd 1986). Some experimental evidence has been obtained to support spatial separation, as both functions occurred during continuous illumination in the mycelial network of *Pisolithus tinctorius* and *Suillus bovinus* mycorrhizas on *Pinus* spp. and *Betula pendula* (Boyd 1987).

2.3.2 Effects of drought and other environmental stresses on mycorrhizal formation

The basic mechanisms of ectomycorrhizal formation are not well understood, and since Björkman (1942) published his theory on the decisive role of low N (and P) levels in plants leading to 'surplus' carbohydrates in roots which make it possible for mycorrhizal fungi to colonize them, there has been much controversy about the subject (Marx *et al.* 1977, Nylund 1988). A second major theory has been promoted by Slankis (1973,1974), suggesting that fungal auxins increase transport of carbohydrates to roots. Hence the high levels of soluble carbohydrates found in mycorrhizal roots would be the consequence rather than the cause of infection, and the inhibition of mycorrhizal formation by high nutrient levels would be an interaction between N and hormones: high N concentrations suppress formation of fungal auxins (Slankis 1973). This theory has been increasingly supported by experimental data (Mudge 1987, Nylund

1988, Nylund & Wallander 1989).

The effect of a particular environmental factor on mycorrhizal formation may be mediated in different ways depending on the host and fungal species involved, their developmental stage and environmental conditions. Therefore the effects of environmental factors must be considered in relation to the whole system involved. For example, soil temperature influences the whole pattern of root development, including long root elongation and short root initiation. Hence the number of short roots available for infection is affected as well as the growth of the fungus in the rhizosphere, and these changes may result in an altered pattern of mycorrhizal formation (Wilcox & Ganmore-Neumann 1975). The number of short roots available for infection may vary between genotypes such that this has been proposed as a reason for delayed mycorrhizal colonization in Douglas fir subspecies (Miles & Antibus 1987). However, Reid (1979) referred to unpublished results by M. Cline and C.P.P. Reid showing that even though the proliferation of short roots was under strong genetic control in *Pinus ponderosa* Laws. and *P.flexilis*, soil moisture significantly affected short root numbers in some seed sources.

The physiological status of the host plant is dependent on the environment, and so are rates of processes such as carbohydrate translocation and root exudation, which affect mycorrhizal formation (Theodorou & Bowen 1971). Root exudation of *Pinus ponderosa* seedlings under water stress was increased when the substrate water potential fell to -0.19 MPa, then decreased in -0.55 MPa, and increased in -1.2 MPa. The composition of the exudate was affected by water stress, too, the proportion of sugars relative to organic acids increasing (Reid 1974). Further, Reid & Mexal (1977) found an increase in *Pinus contorta* exudation from 0 to -0.2 to -0.4 MPa, but as the total assimilate allocated in roots increased with increasing stress, the proportion of exuded substances was largest at 0 MPa.

As already indicated, the best known environmental effect on mycorrhizal formation is that of nutrient availability. Mycorrhizal formation can readily be inhibited by applying high levels of nitrogen to the rooting medium in the laboratory (Slankis 1973). Colonization of containerized coniferous seedlings has frequently been found to be inhibited by high nutrient concentrations (Anttila & Lähde 1977, Ruehle 1980, Shaw *et al.* 1982, Ruehle & Wells 1984, Danielson *et al.* 1984b), but not in the case of all fungi: *Laccaria laccata* (Molina

& Chamard 1983) and an ectendomycorrhizal fungus (Danielson *et al.* 1984b) colonized root systems equally efficiently over a range of fertilizer levels tested.

Early work on mycorrhizal infection showed that low light levels inhibit colonization (Björkman 1942), and this result has been repeated more recently (Reid *et al.* 1983, Ekwebelam & Reid 1983). Elevated CO_2 atmosphere has similarly been found to increase infection (O'Neill *et al.* 1987). This is most probably due to the effects of light and CO_2 on photosynthetic activity and hence carbon availability to the fungus. Shading under natural conditions did not impede mycorrhizal formation of 5-year-old *Picea abies*, possibly because seedlings had access to carbohydrate supplies of adjacent large trees through interconnections of mycelial strands (Kottke & Oberwinkler 1986).

Air pollutants can also reduce mycorrhizal formation, probably by their effects on the condition of the host plant. Both simulated acid rain and relatively high ozone concentrations have reduced the mycorrhizal proportion in *Quercus rubra* (Reich *et al.* 1985) and *Pinus strobus* (Stroo & Alexander 1985, Stroo *et al.* 1988) even if the growth of seedlings has not been affected much by the treatment.

The amount of organic matter in soil is often mentioned as a factor promoting mycorrhizal infection (Göbl & Platzer 1967, Meyer 1974), but long-term experiments in British nurseries showed no effect of various organic amendments on mycorrhizas (Levisohn 1965, Benzian *et al.* 1972, Low & Sharpe 1973).

Theodorou and Bowen (1971) found that growth and mycorrhizal development by various fungi on *Pinus radiata* (D.) Don. was remarkably reduced by a decrease of temperature from 25°C to 20°C to 16°C. On the other hand, high temperatures inhibit mycorrhizal formation as well, different fungi having different temperature optima: *Pisolithus tinctorius* formed mycorrhizas on *Pinus taeda* best at 34°C, whereas *Thelephora terrestris* formed hardly any at this temperature (Marx *et al.* 1970).

Ectomycorrhizal fungi are highly aerobic (Harley & Smith 1983), and flooding has been reported to reduce mycorrhizal formation in nursery soils (Morby *et al.* 1978) as well as peatlands (Heikurainen 1955, Paavilainen 1966). Boyd (1987) found fewer *Pisolithus* mycorrhizas on *Betula* in peat kept at field capacity than

in 70 % or 30 % moisture content of field capacity. Similarly, mycorrhizal formation by *Rhizopogon luteolus* and unidentified soil fungi on *Pinus radiata* was depressed in soil moisture contents approaching saturation (Theodorou 1978).

Shemakhanova (1962, cited by Reid 1979) and Shemakhanova & Mazur (1968, cited by Reid 1979) found that formation of mycorrhizas on oak and hazelnut was strongly influenced by water availability in the field. Similarly, the colonization of *Pinus virginiana* Mill. by native soil fungi was decreased to almost nil by drying of the soil in pot experiments (Worley & Hacskaylo 1959). Theodorou (1978) found that the growth of *Rhizopogon luteolus* and *Cenococcum graniforme (C. geophilum)* in the rhizosphere had optimum soil water potentials of -0.06 to -0.12 MPa and -0.06 MPa, respectively. Both below and above this water regime the relative length of root colonized was lower. He attributed the depression of growth in low soil water potentials to diminishing exudation of energy substances from the pine roots, since the fungi were able to grow in much more severe water stress if they were provided with glucose and yeast extract.

2.3.3 Water stress and fungi in pure culture

As the growth of fungal hyphae occurs in response to a turgor pressure gradient, it is likely to be reduced by stress induced by a decrease in the osmotic or matric potential of the substrate (Eamus & Jennings 1986).

Mexal & Reid (1973) found that *Cenococcum graniforme (C. geophilum* had a maximum growth rate in -1.5 MPa induced with polyethylene glycol, whereas *Suillus luteus* (Fr.) S.F.Gray and *Thelephora terrestris* had maxima in -0.5 MPa. Theodorou (1978) showed considerable differences between five ectomycorrhizal fungi in soil amended with glucose and yeast extract in their resistance to low osmotic potentials. The lowest potentials in which mycelial growth was possible were remarkably low compared to those which inhibit the growth of plants: -4 MPa for *C. geophilum* and *Suillus luteus*. Coleman *et al.* (1989) also showed differences between species, even though in some cases these were overridden by differences between isolates of the same species. They named eight species out of 18 as drought tolerant *(Boletus edulis* Bull.: Fr., *Cenococcum geophilum, Rhizopogon vinicolor* and five *Suillus* spp.) on the

basis of their growth rates at -3 MPa, and some species as intolerant (*Hebeloma crustuliniforme* (Bull. ex St.Amans) Quél., *Laccaria bicolor* (Maire) Orton, *L. laccata* (Scop.: Fr.) Berk. & Br. and *Suillus caerulescens* Smith & Thiers.) as these did not grow at water potentials lower than -1 MPa.

However, as pointed out by Reid (1979), if a fungus can grow in a very low ambient water potential, it does not necessarily follow that mycorrhizal structure would be beneficial for the host, because the water potential of the fungal partner needs to be less negative than that of the plant to support water flow into the plant.

Even though the growth rates of different isolates of the same fungus in laboratory media are sometimes correlated with the ability of the fungus to form mycorrhizas in nonstressed conditions (Graham & Linderman 1981), attempts to relate the stress resistance of fungi in pure culture and in the rhizosphere have not always been successful. Part of the problem is that water stress is usually induced in laboratory media by using osmotica, and the resulting condition is different from low soil matric potential. Theodorou (1978) found little correlation between the growth of a particular fungus in pure culture subjected to osmotic stress and colonization of *Pinus radiata* roots by the same fungus at low water potentials. Parke *et al.* (1983) came to the same conclusion, referring to their unpublished results on pure cultures of different fungi, which were not consistent with results of experiments on the drought resistance of Douglas fir seedlings inoculated with the same fungi, even though the results of Coleman *et al.* (1989) on pure culture growth were better correlated to the results reported by Parke *et al.* (1983). Similarly, the temperature optima of fungal isolates on laboratory media have been found to be different from their optimum temperatures for growth in the rhizosphere (Theodorou & Bowen 1971). Therefore it seems to be of limited value to study the stress responses of fungi in pure culture unless there is some interest in these fungi as such rather than in symbiosis.

2.3.4 Water uptake by extraradical strands

Many ectomycorrhizal fungi - but not all - form an extensive network of hyphae into the surrounding soil. This mycelium may consist of a weft of individual hyphae, or it may be highly organized into strands. The structure of

strands may involve larger 'vessel' hyphae of diameters of 6–20 µm, which generally lack cytoplasmic contents, surrounded by narrower, densely cytoplasmic hyphae often with thick cell walls as in strands of *Suillus bovinus* (Duddridge *et al.* 1980), and *Paxillus involutus, Lactarius rufus* (Fr.) Fr. and *Leccinum scabrum* (Fox 1983). Read & Malibari (1979), using tritiated water, showed that strands of *Suillus bovinus* took up water and transported it to roots of Scots pine seedlings. Subsequently, Duddridge *et al.* (1980) measured minimum rates of translocation of tritiated water from strands to pine shoots of 27 cm h^{-1}, which is of the same order as rates measured for transport in xylem.

Experiments on Scots pine and birch *(B. pendula)* plants with mycelial strands of *Suillus bovinus* extending to moist peat whilst the root systems were in dry peat, demonstrated that strand systems can supply seedlings with enough water to keep them alive; when the strands were severed, the transpiration and photosynthetic rates decreased rapidly, and the plants died of dehydration (Boyd *et al.* 1986, Read & Boyd 1986). In the same types of experiments, Brownlee *et al.* (1983) found that Scots pine seedlings remained in apparently healthy condition for more than ten weeks with mycelial strands in moist peat as the only source of water.

2.3.5 Responses of mycorrhizal and nonmycorrhizal plants to water stress

A number of reports have been published on experiments comparing the reactions of ectomycorrhizal and nonmycorrhizal plants to drying soil, usually testing the hypothesis that mycorrhizal plants take up water more efficiently during drought and hence can maintain their stomatal opening longer without a decrease in water status. However, the results have been somewhat conflicting, and mycorrhizal plants have not always performed better during a drought treatment.

Sands and Theodorou (1978) were the first to experimentally study the water relations of ectomycorrhizal *(Rhizopogon luteolus)* and nonmycorrhizal seedlings. They found no effect of mycorrhizas on the water relations of *Pinus radiata* seedlings when soil moisture was adequate, but during drought the leaf water potentials were more negative for mycorrhizal plants. Consequently, the resistance of plant plus soil was higher for mycorrhizal plants, but Sands &

Theodorou still considered the soil component of the combined resistance as more important to water flow than the plant component, hence the difference between mycorrhizal and nonmycorrhizal plants could have been due to differences in root geometry. Sands *et al.* (1982) subsequently measured hydraulic conductance of mycorrhizal (mainly *Pisolithus tinctorius)* and nonmycorrhizal *Pinus taeda* roots, and found no effect of mycorrhizal infection, but a substantial effect of suberization on the conductance. More recent studies have failed to show a greater conductance to the flow of water in ectomycorrhizal than nonmycorrhizal plants or root systems as well. Diebolt & Mudge (1987) found no difference in the transpiration and predawn water potential of *Pisolithus tinctorius*-mycorrhizal and nonmycorrhizal Scots pine. In an experiment comparing Douglas fir either heavily mycorrhizal with *Laccaria* or *Hebeloma* sp. and controls with lower mycorrhizal percentage, Coleman *et al.* (1987) found a higher root hydraulic conductance in the control seedlings, even though the mycorrhizal plants had higher root phosphorus concentrations, and P fertilization as such increased root conductance.

Nevertheless, mycorrhizas have improved seedling water relations in many cases. Dixon *et al.* (1980) reported higher water potentials in irrigated *Pisolithus tinctorius*-mycorrhizal white oak *(Quercus alba* L.) than nonmycorrhizal, growing in root observation containers. During a drought treatment the larger mycorrhizal plants dried the soil out more efficiently, yet their leaf conductances and water potentials were not significantly lower than those of nonmycorrhizal plants. Also, their water potentials and leaf conductances recovered to pre-stress values sooner after rewatering. The mycorrhizal plants had more extensive root systems, and their root elongation rates were higher during the drying cycle, which probably accounted for their recovery. Dixon *et al.* (1980) referred to results by Shemakhanova, which indicated improved recovery of ectomycorrhizal oak after irrigation. Moreover, in a field experiment black oak *(Quercus velutina* Lam.) inoculated with *Pisolithus* had higher water potentials and lower soil-to-plant resistance than nonmycorrhizal consistently over the growing season (Dixon *et al.* 1983).

Boyd (1987) also found that mycorrhizal infection by *Pisolithus tinctorius* increased the transpiration rates and water potentials, and hence the soil-plant conductance to water in birch *B. pendula* plants exposed to moderate water stress. As the plants were of comparable size, and their shoot phosphorus concentrations were not different, Boyd maintained that the increased

conductance was caused either by the fungal mycelium extending the potential volume of soil exploited by a mycorrhizal plant, and also, it may have improved the contact between the peaty substrate and root. In this experiment, the plants were grown in different water regimes for a relatively long time, 42 or 63 days.

In another long-term experiment on mycorrhizal effects on drought resistance, Parke *et al.* (1983) subjected mycorrhizal (unidentified soil fungi) and nonmycorrhizal Douglas fir seedlings to several drying and rewetting cycles, and subsequently measured net photosynthetic rates ten times higher in mycorrhizal plants, even though their average water potential was considerably lower than that of nonmycorrhizal plants. However, in another experiment with a suddenly imposed drought treatment they measured a greater difference in the photosynthetic and transpiration rates between plants inoculated with different mycorrhizal fungi than mycorrhizal and nonmycorrhizal plants during drought and recovery. Unfortunately they did not report nutrient concentrations of plants, which might have partly explained the results of the first experiment. These two experiments indicate that mycorrhizas were more important in long-term drought resistance than in short-term.

One of the proposed explanations to the relatively low shoot water potentials in mycorrhizal plants in some experimental conditions is more efficient osmotic adjustment, caused by mycorrhizal effects on plant mineral and carbon nutrition (Parke *et al.* 1983). Pallardy *et al.* (1983) tested this hypothesis by performing pressure-volume analysis on shoots and root systems of shortleaf pine *(Pinus echinata* Mill.) mycorrhizal with *Pisolithus tinctorius,* or nonmycorrhizal. Their results did not show any mycorrhizal effect on tissue water relations, except for a larger mean osmotic volume for mycorrhizal plants, which was attributed to their much larger size. Both mycorrhizal and nonmycorrhizal seedlings adjusted osmotically to drought. However, mycorrhizal (unidentified fungi) *Gmelina arborea* Roxb. seedlings did adjust osmotically more efficiently than nonmycorrhizal, which enabled them to maintain their turgor in a drier soil, and keep their stomata longer open (Osonubi 1989). This effect was attributed to the much higher phosphorus status of the mycorrhizal plants and their larger root growth.

Only three of the studies referred to above included measurements of host plant nutrient concentrations, even though it is well-known that mycorrhizas

influence nutrient uptake from soil, and plant nutrition influences plant water relations. More attention to the interaction of nutrition, water relations and mycorrhizas has been paid in the work done on VA mycorrhizal plants, which is briefly discussed in Section 2.4. Moreover, most of the studies on both ecto- and VA mycorrhizal water relations are concerned with short-term effects of drought, and hence ignore the possible mycorrhizal effects on sustained growth and water and nutrient uptake in fluctuating conditions.

2.3.6 Mycorrhizas formed by different fungi in relation to water stress

Worley and Hacskaylo (1959) were among the first to report changes in mycorrhizal populations of *Pinus virginiana* as a result of droughting. They found that the proportion of *Cenococcum geophilum* increased relative to the dominant white mycorrhizal type, but this was a result of a major decrease in the numbers of the other type whilst the numbers of *Cenococcum* did not decrease as much. This fungus has also been able to grow at low substrate water potentials in pure culture (Section 2.3.3., Saleh-Rastin 1976). However, evidence of the effects of *Cenococcum* on the drought resistance of host plants is lacking. Pigott (1982) attributed the dominance in the field of *Cenococcum* mycorrhizal root tips of *Tilia cordata* Mill. during dry conditions to better survival of these mycorrhizas rather than improved water uptake, hence they would be of benefit after mitigation of the drought rather than during drought.

In the experiments of Parke *et al.* (1983) into short-term drought resistance of different mycorrhizal Douglas fir seedlings, plants inoculated with *Rhizopogon vinicolor* had much higher gas exchange rates than nonmycorrhizal plants or plants inoculated with *Laccaria laccata, Pisolithus tinctorius,* or an unidentified fungus during a drought treatment and recovery. They speculated on the reasons for the superiority of this fungus, and proposed that it was caused by the abundant, well-organized strands of *Rhizopogon* mycorrhizas. However, the soil or plant water status was not assessed in this experiment which was carried out in small pots. As the *Rhizopogon* mycorrhizal plants were smaller than plants in other treatments, their higher gas exchange rates might have been due to higher soil moisture content as well as higher water uptake rates from dry soil.

The ability of *Pisolithus tinctorius* to improve seedling performance as opposed to forest tree nursery fungi and other native soil fungi is well established, and it is thought to be in part a result of improved water relations of the host (Marx *et al.* 1984). The effect of *Pisolithus* on plant water relations was shown in a field experiment on a coal spoil by Walker *et al.* (1983), in which *Pisolithus* -inoculated *Pinus virginiana* had significantly higher water potentials than seedlings mycorrhizal with endemic nursery fungi. Similarly, the water potentials of *Pinus taeda* with *Pisolithus tinctorius* were higher compared to seedlings with native fungi on the third growing season after outplanting (Walker *et al.* 1989). The nitrogen status of these seedlings was also higher, and the leaf Zn was lower. However, in some pot experiments *Pisolithus* has failed to improve host water relations compared to other inoculants (Parke *et al.* 1983).

2.4 Vesicular-arbuscular mycorrhizas and plant water relations

Vesicular–arbuscular (VA) mycorrhizas have often been reported to increase transpiration or stomatal conductance of the host plants as opposed to nonmycorrhizal plants both in watered (Safir *et al.* 1971, Allen *et al.* 1981, Allen 1982, Huang *et al.* 1985, Augé *et al.* 1986a, Hardie & Leyton 1981) and dry soil conditions (Levy & Krikun 1980, Allen & Boosalis 1983, Augé *et al.* 1987) without concomitantly decreased plant water potentials, and enhance yields during drought (Hardie & Leyton 1981, Busse & Ellis 1985, Bethlenfalvay *et al.* 1988). More extensive osmotic adjustment has also been found in VA mycorrhizal plants (Allen & Boosalis 1983, Augé *et al.* 1986b), as well as increased root hydraulic conductance (Safir *et al.* 1972, Hardie & Leyton 1981, Graham & Syvertsen 1984).

Several hypotheses have been put forward to explain the improved water relations of mycorrhizal plants: increased nutrient uptake, increased water uptake by means of either larger root systems or fungal hyphae, and hyphae within the root providing a high conductance pathway for water movement (Safir & Nelsen 1985). As phosphorus deficiency is known to decrease the stomatal opening and root hydraulic conductance (Section 2.2.), and nonmycorrhizal plants tend to have lower P concentrations than VA mycorrhizal, it has been a subject of controversy, whether there is any

nonnutritional effect of mycorrhizal infection on plant water relations (Safir & Nelsen 1985, Graham 1987). Safir *et al.* (1972) tested this by applying fungitoxicant in mycorrhizal soybean *(Glycine max* (L.) Merr.) which had higher stomatal conductance compared to nonmycorrhizal plants, and as this did not affect the resistances to water transport, they concluded that the mycorrhizal effect was not caused by direct modification of the pathway. Further, they found that application of high nutrient levels essentially eliminated the mycorrhizal effect. Since then, other studies have shown that increased root conductance (Graham & Syvertsen 1984, Graham *et al.* 1987, Andersen *et al.* 1988) or stomatal conductance (Fitter 1988) in VA mycorrhizal plants does not occur unless there is also an increase in phosphorus nutrition. However, Augé *et al.* (1986a) argued that there was an intrinsic difference in water relations of watered mycorrhizal and nonmycorrhizal *Rosa hybrida* L., as low-P mycorrhizal plants had higher leaf conductances than either high-P mycorrhizal and nonmycorrhizal, leaf water potentials being similar in all treatments. Further, they found higher leaf conductance accompanied with higher water in mycorrhizal rose plants exposed to drought regardless of P regime (Augé *et al.* 1987).

Allen *et al.* (1981) suggested that the higher stomatal conductances of VA mycorrhizal *Bouteloua gracilis* (H.B.K.) Lag ex Steud were associated with a change in the hormonal levels, possibly cytokinins originating in the roots and transported to leaves. Higher cytokinin concentrations have been measured in leaves of mycorrhizal than nonmycorrhizal plants (Allen *et al.* 1980, Dixon *et al.* 1984). However, plants with adequate P concentrations are also known to have higher leaf cytokinin contents than P deficient plants (Radin 1984, Baas & Kuiper 1989).

One possible way in which VA mycorrhizas may improve the ability of the plant to acquire water, is increased absorbing surface area by extraradical hyphae. Allen (1982) calculated that the increased water flux in mycorrhizal *Bouteloua* could be accounted for by the number of hyphae found, and on the basis of water transport rates reported for other fungi. However, Graham & Syvertsen (1984) concluded after similar calculations that hyphae could not account for all of the additional water uptake by their mycorrhizal plants. Safir & Nelsen (1984) pointed out that several assumptions in this type of analysis may not be warranted even though it may yield information on hyphal water uptake. Hardie (1985) used a more direct approach to study the water uptake by VA

mycorrhizal hyphae associated with *Trifolium pratense* L. and *Allium porrum* L. When extraradical hyphae were severed, the water uptake of the plants was significantly decreased relative to intact controls, and she concluded that there is a mycorrhizal effect on water uptake independent of nutrition. This could conceivably lead to mycorrhizal plants being able to take up water at lower soil potentials and hence wilt in drier conditions than nonmycorrhizal (Hardie & Leyton 1981).

The results from these and other experiments on VA mycorrhizal and nonmycorrhizal plants and their water relations are conflicting, even though it is clear that if conditions are such that mycorrhizas improve nutrient uptake, they also have an effect on water relations. It is likely that there are several ways for the mycorrhizal infection to affect the physiology of the plant, and these are interdependent, not mutually exclusive (Read & Boyd 1986).

CHAPTER 3
GENERAL MATERIALS AND METHODS

3.1 Production of plant material

3.1.1 Preparation of substrates

Sitka spruce *(Picea sitchensis* (Bong.) Carr.) seedlings for each experiment were produced in semiaseptic conditions, using either autoclaved vermiculite-peat mixture or acid washed perlite (expanded volcanic rock) as a medium. Both media have been widely used in mycorrhizal experimentation, as good mycorrhizal formation and growth of plants can be obtained in them.

Vermiculite-peat was prepared by mixing horticultural vermiculite (medium grade Vermiperl, Silvaperl products, Harrogate) and ground moss peat in proportions of 480 g vermiculite to 300 g peat. The mixture was moistened with 2.9 l water and autoclaved at 121°C and 0.103 MPa for 30 minutes.

Perlite was chosen for some experiments because it is, after treatment, a relatively inert substrate, allowing control of plant nutrition. Acid washing has been found to reduce the total amount of exchangeable cations to almost half, and to reduce the pH of Ingestad solution for birch on perlite from 4.95 to 4.43 (Denny 1986). Therefore horticultural perlite (standard grade Silvaperl, Silvaperl products, Harrogate) was acid washed before use by soaking it overnight in 0.2 M nitric acid, mixing at intervals. The next day the acid was drained off and the perlite was rinsed in three changes of tap water, then soaked in 0.1 M ammonium hydroxide for 4 hours, rinsed, and oven dried at 80°C (Denny 1986).

3.1.2 Fungal inocula

The mycorrhizal fungi used in experiments were obtained from the Institute of Terrestrial Ecology culture collection at Bush Estate.

The species used were:

Paxillus involutus (Batsch) Fr. *(Boletales, Boletaceae)*
Hebeloma crustuliniforme (Bull.: St Amans) Quél. *(Agaricales, Cortinariaceae)*
Laccaria proxima (Boud.) Pat. *(Agaricales, Tricholomataceae)*
Thelephora terrestris (Ehrh.) Fr. *(Aphyllophorales, Thelephoraceae)*

The fungi had been isolated from the following sources:

Paxillus involutus from a fruitbody on coal waste in Midlothian, with 15-20 years old *Betula pendula*, in 1982
Hebeloma crustuliniforme from a fruitbody on a brown earth with 5 years old Sitka spruce at Bush Estate, in 1983
Laccaria proxima reisolated from a fruitbody in a pot experiment on 2 years old Sitka spruce at Bush Estate in 1982
Thelephora terrestris, a root isolate from Sitka spruce ex R. Jackson, University of Surrey, in 1982.

Modified Melin-Norkrans medium (MMN; Mason 1980) was used for growing fungi in flasks. This differs from MMN as specified by Marx (1969) in that glucose is used rather than sucrose and malt extract. The inocula were produced in aseptic conditions either in liquid culture with modified Melin-Norkrans medium or in a vermiculite-peat mixture moistened with MMN (25 g vermiculite, 6 g peat, 180 ml MMN).

3.1.3 Cultivation of Sitka spruce seedlings

Sitka spruce seeds of Queen Charlotte Island provenance, Skidegate, were obtained from the Forestry Commission, Northern Research Station, Bush Estate. The same seedlot was used to produce plants for all experiments.

Seeds were surface sterilized by soaking for 12 h in 0.01 % thiram solution, and kept moist in a cold room at 3°C for 3 weeks. Polythene pots used in experiments 1,2,3 and 4 were sterilized by soaking in sodium metabisulphite for two days. Pots were filled with autoclaved vermiculite-peat or acid washed perlite on the laboratory bench, which was cleaned with industrial alcohol, as were all tools used. After sowing the seeds the pots were moved to either a glasshouse or growth room. When first lateral roots had emerged, usually about five weeks from germination, the plants were inoculated with a piece of

mycelium grown in liquid culture or with a piece of vermiculite-peat with mycelium from a vermiculite-peat culture. The inoculum was placed at the middle part of the root system, next to short roots if any were visible. The inoculation was done in semiaseptic conditions in the same way as for sowing seeds.

3.1.4 Nutrition

A nutrient solution modified from Ingestad solution for birch (Ingestad 1979) to meet the requirements of Sitka spruce for optimum growth (Van den Burg as quoted by Ingestad 1979; Cape, N., peronal communication) was used to feed the plants in all experiments. The proportions of N, P, and K were 100:16:55, and the ratio of NO_3-N to NH_4-N was 7:5. The stock solutions A and B were prepared as follows.

Solution A		Solution B	
	g l^{-1}		g l^{-1}
NH_4NO_3	140.2	HNO_3	1.6
KNO_3	37.2	H_3BO_3	0.57
KH_2PO_4	41.3	$Fe_2(SO_4)_3$	2.5
K_2SO_4	14.0	$Ca(NO_3)_2$	14.3
		$Mg(NO_3)_2$	26.0
		$MnSO_4$	0.55
		$CuCl_2$	0.032
		$ZnSO_4$	0.036
		$NaMoO_4$	0.007

Stock solutions were mixed in the following proportions: 1.7 parts solution A to 1.0 parts solution B. The strength of the solution used for feeding was expressed in mg N l^{-1}.

3.2 Assessment of mycorrhizal formation

For assessment of mycorrhizal formation, root systems were gently washed under running water and then placed in Petri dishes in water for examination of the tips of all short roots. A short root was defined as a root which was not branched, hence the category also includes relatively 'long' roots particularly in

nonmycorrhizal root systems. The tips were categorized according to whether they were mycorrhizal or not. A mycorrhizal root tip was defined as a short root with a mycelial sheath, however thin. Short roots exhibiting thickening and check of growth characteristic of ectomycorrhizas and possibly a Hartig net were not counted as mycorrhizas, if they did not have a sheath.

When closer examination was necessary, to confirm the mycorrhizal status or the fungal species, short roots were squashed under a cover slip with 1 % cotton blue in 10 % lactophenol and their sheath and hyphal characteristics were inspected under a high-power microscope (Ingleby *et al.* 1989). In this way the Hartig net could be observed in the short roots as well; usually this was present when the sheath was only beginning to form.

3.3 Transpiration by gravimetric method

Transpiration rates were determined from the difference in weight before and after a four hours period in a growth room. The surface of the pots was covered with 'Parafilm M' (American Can Company, Greenwich, Connecticut) to prevent evaporation from the substrate. In preliminary experiments 'Parafilm' was found to be superior to 'Clingfilm' or polythene, yet some evaporation (0-2 mg h^{-1}) was measured from control pots with a wooden stick instead of a plant. Therefore such controls were run parallel to Experiment 1b, with as many pots per water regime as there were replicates per treatment. The means of evaporation rates were calculated for each set of measurements, and the mean was subtracted from the difference between the two weight measurements of each pot.

3.4 Gas exchange by an IRGA and dewpoint meter system

3.4.1 Measuring system

The measuring system consisted of two infrared gas analyzers (IRGA, model SB2, Grubb Parsons, Newcastle) which were used in absolute and differential modes, and a dewpoint hygrometer (model 880, EG & G, Cambridge).

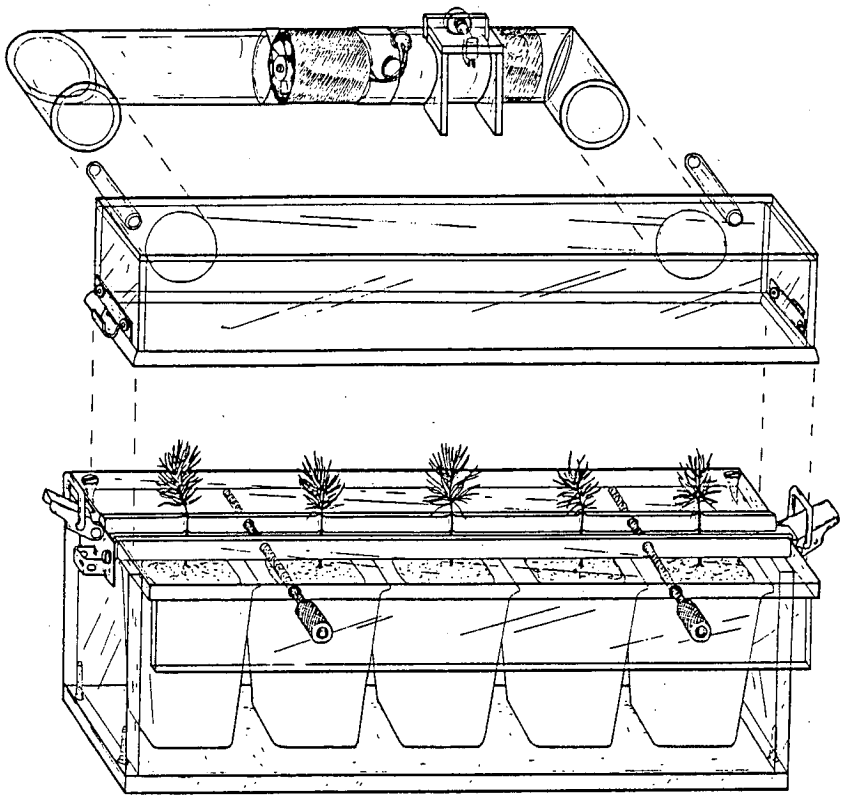


Fig. 3.1. Sitka spruce seedlings in a leaf chamber (Section 3.4.)

The leaf chamber (Fig. 3.1.), accommodating four or five small seedlings, was made of 'Perspex'. Air was stirred with a miniature fan (Radiation Components, Twickenham). The light levels within the chamber were determined using a home-built light meter with a general purpose photodiode, which was calibrated against a Li-Cor model LI-190S-1 (Li-Cor, Lincoln, Nebraska) quantum sensor. The chamber and needle temperatures were measured with thin copper-constantan thermocouples and recorded at 10 min intervals on a chart recorder.

The air flow in the system is shown schematically in Fig. 3.2. Air was drawn in from a rain-proof entry point on the roof with a Reciprotor pump (P in Fig. 3.2.) into two 182 litre containers in series which acted as homogenizers (H1,H2). The air was then humidified in two pairs of large gas bubblers with sintered glass disks in water bath 1 (WB 1), which was held at least at the temperature of the growth room where the plants were during the measurements, by means of an immersion heater (I). Before entering the humidifiers the air passed through copper coils to raise the temperature to the water bath temperature.

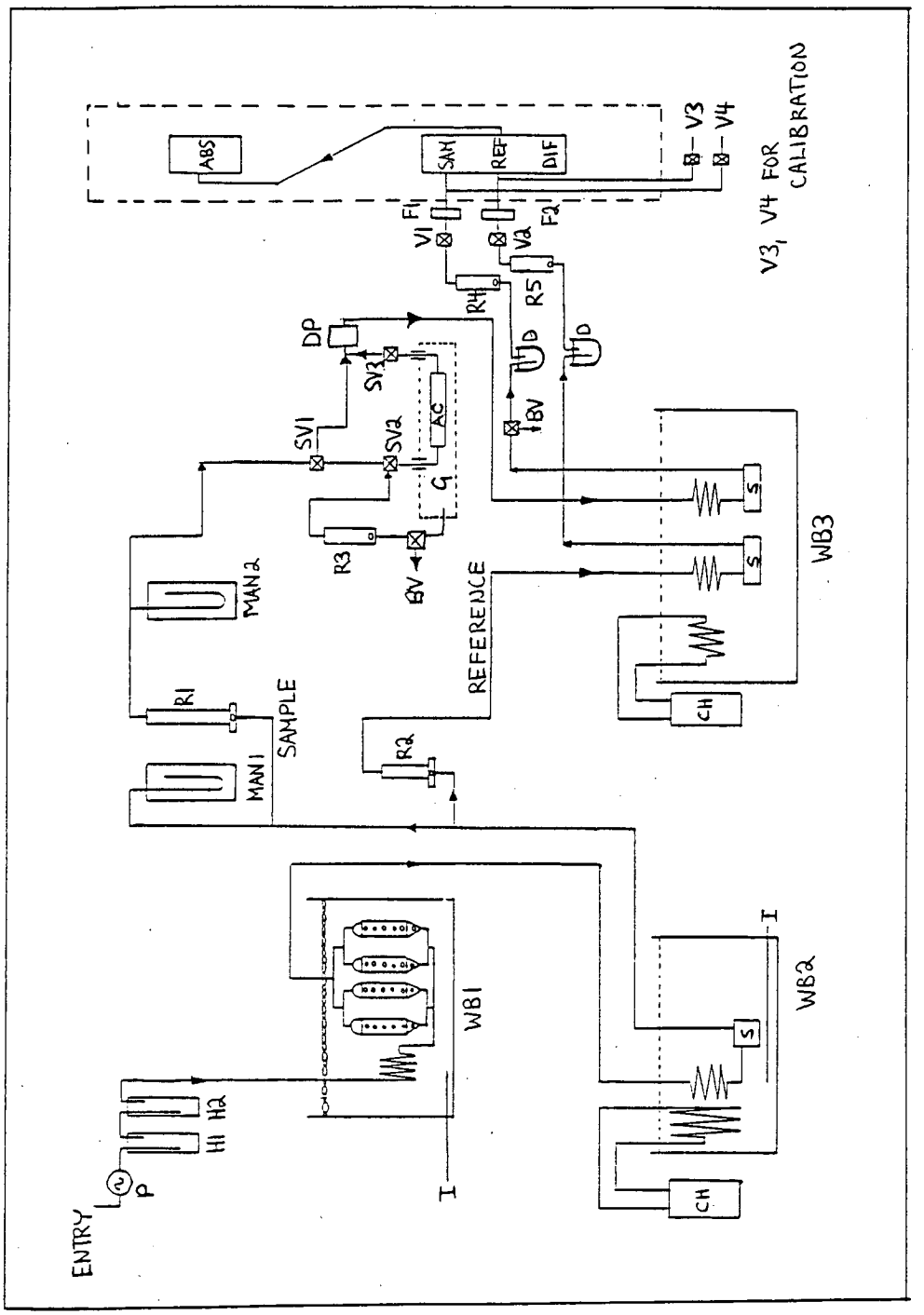


Fig. 3.2. Air flow in the system for measuring gas exchange (Section 3.4.1.). See text for annotations.

Excess moisture was then condensed out in a copper condenser coil with a sump (S) for condensed water in water bath 2 (WB2), kept about 3 °C below the dewpoint temperature of the growth room by a chiller (CH; type RCU3, F&R Cooling, Wellington, Somerset) or a heater (I). The airstream was split into reference and sample lines. The pressure on line was checked on manometer 1 (Man 1), the flow rates were then controlled by 'Rotameter' flow controllers R1 and R2 (KDG Instruments, Croydon, Surrey), and the lowered pressure was observed in manometer 2 (Man 2), and maintained near 101.3 kPa. The sample line either passed through the leaf chamber (AC) in the growth room (G) or was bypassed for background measurements by switching solenoid valve SV1 respectively. During background measurements the chamber was flushed with air taken from the growth room, the flow being directed by solenoid valves SV2 and SV3, and regulated by 'Rotameter' R3 and bleed valve BV. This enabled changing the plants in the leaf chamber and closing this during the background measurements without the air CO_2 and H_2O concentrations within the chamber changing too much.

After the dewpoint hygrometer (DP) on line, part of the moisture was condensed in copper condensing coils with sumps for excess water in water bath 3 (WB3, temperature between 3...7 °C), and the remaining moisture was removed by passing the stream through two columns of magnesium perchlorate (D) which was changed daily. Solid particles were removed by Whatman 41 filter paper (F1,F2). Before the IRGAs, flow rates of the two lines were balanced with 'Rotameter' flowmeters R4 and R5. Deflection of the differential IRGA was recorded on a chart recorder. The IRGAs were enclosed in a cabinet to hold the temperature nearly constant. The temperature of the IRGAs was recorded automatically on a chart recorder.

The tubing used was 'Dekabon 1300' (Dekoron Division, Samuel Moore Europe, Coventry) tubing, diameter 6.25 mm, except before water bath 1, where nylon tubing was used.

Atmospheric pressure was measured with an electronic aneroid barometer (Penny & Giles Transducers, Christchurch, England) and monitored continuously.

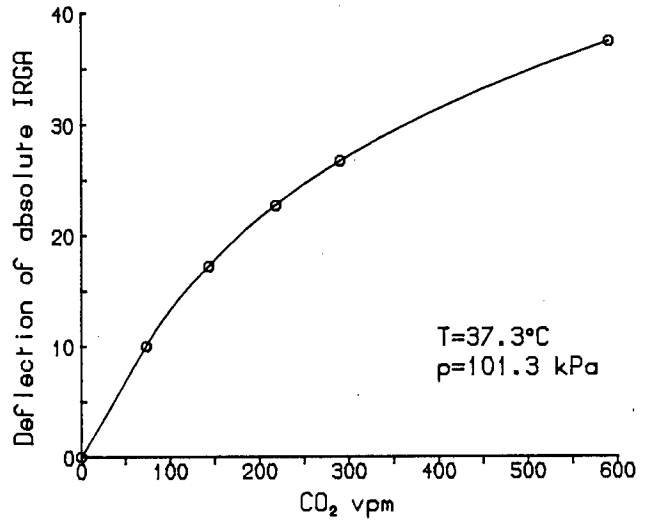
3.4.2 Calibration of IRGAs

The absolute and differential IRGAs were calibrated before each set of measurements, which each consisted of one determination of the gas exchange of 16 sets of plants (Chapter 4), and took 2 or 3 days. The differential IRGA was calibrated each day before the measurements commenced. A gas mixing system was used to obtain suitable concentrations of CO_2 by mixing N_2 gas with dry gas of known CO_2 concentration from a commercial gas cylinder. As a check, the concentration of the commercial gases was also measured with another IRGA (ADC, Hoddesdon, Herts.) calibrated as described in Section 3.5.2. These results did not deviate from each other enough to give reason for corrections to the concentrations given by the supplier. The zero point of the absolute IRGA was calibrated using N_2 gas. Typical calibration curves are shown in Fig. 3.3., a and b. These were used for transforming the readings from IRGAs to CO_2 concentrations, which were corrected for the differences in the temperatures of the IRGAs and the atmospheric pressure during calibration and the experiment.

3.4.3 Determination of H_2O and CO_2 exchange

The equipment was set up to obtain a steady value for background readings of the differential IRGA and the dewpoint meter. Four seedlings were sealed into the leaf chamber for each determination (Fig. 3.1.). A fine thermocouple was attached under a needle, parallel to it, using adhesive obtained from 'Sellotape' dissolved in chloroform. The flow rate to each side of the IRGA was balanced to 400 ml min^{-1} as the IRGAs were calibrated at this flow rate. Readings of absolute and differential CO_2 concentrations and dewpoint temperature were taken for the background and sample airstreams. For each sample, the needle, leaf chamber, IRGA and 'Rotameter' temperatures, and atmospheric pressure were recorded. Readings were taken when the differential IRGA was recording a steady CO_2 concentration which took 15–20 min from the time the leaf chamber was closed for the sample readings and about 5 min for the background readings.

a



b

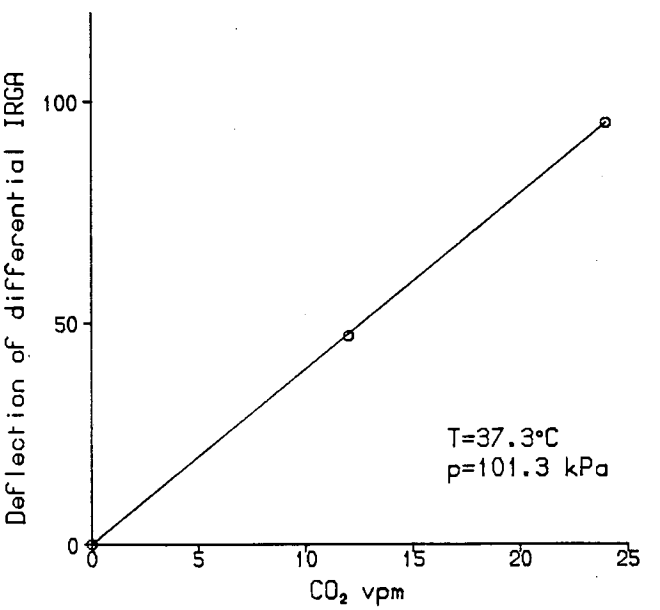


Fig. 3.3. **a** Calibration curve for the absolute IRGA
b Calibration line for the differential IRGA.

3.4.4 Calculations

The calculations are based on Šesták *et al.* (1971) and Anon. (1988). The boundary layer resistance (r_b) of Sitka spruce shoots used was 1.01 m^2 s mol^{-1} (determined by E.J. White)

The variables obtained from measurements were

J = flow rate ml s^{-1}
a = leaf area cm^2
dc = CO_2 concentration difference between the sample and reference lines µl l^{-1}
dp_1 = dew point downstream of chamber °C
dp_a = dew point of the background air stream °C
t_r = temperature of the 'Rotameters' °C
t_n = needle temperature °C
p = atmospheric pressure kPa
r_b = boundary layer resistance m^2s mol^{-1}.

The saturated water vapour pressures at the dew point temperatures and needle temperatures measured were calculated as by Rosenberg (1974, p. 131):

e_1 = water vapour pressure of the air emerging from the leaf chamber kPa
e_a = water vapour pressure of the background air stream kPa
e_n = saturated vapour pressure at the needle surface temperature kPa

Otner calculated variables were

f = mole flow in the system per unit leaf area corrected to STP mol $m^{-2}s^{-1}$

$$f = \frac{J}{22\ 400} \times \frac{273}{273 + t_r} \times \frac{P}{101.3} \times \frac{10\ 000}{a}$$

A = CO_2 exchange rate µmol $m^{-2}s^{-1}$

$$A = f \times dc$$

E = transpiration rate mol $m^{-2}s^{-1}$

$$E = f \times \frac{e_1 - e_a}{p - e_1}$$

g_l = leaf conductance to water vapour mol $m^{-2}s^{-1}$

$$g_s = f \times \frac{e_1 - e_a}{e_n - e_1} \times \frac{p}{p - e_1}$$

g_s = stomatal conductance to water vapour mol $m^{-2}s^{-1}$

$$g_s = \frac{1}{r_l - r_b}$$

where r_l is the reciprocal of g_l.

3.5 Gas exchange by a portable system

3.5.1 Measuring system

The measuring system (The Analytical Development Company, Hoddesdon, Herts.) consists of a portable IRGA (LCA2), an air supply unit (ASUM2) and a leaf chamber (PLC2) with a Coreci humidity sensor on the line after the chamber. Air was taken in from the roof of the building, mixed in two 25 l flasks in series and dried in two tubes of silica gel before entering the leaf chamber. The components were connected with butyl tubing.

3.5.2 Calibration

The zero of the CO_2 analyzer was calibrated with compressed air with relative humidity of 1–2 %, purged of CO_2 by passing it through a column of soda lime (granular mixture of solid NaOH and $Ca(OH)_2$). Air of known CO_2 concentration was obtained by mixing CO_2 with CO_2 free air in a gas mixing unit. Two different concentrations of CO_2 were used to check the span.

The zero point of the humidity sensor was calibrated using air which had been dried by leading it through a column of magnesium perchlorite, and the span was checked with air of known humidity obtained by first humidifying it in a tube with wet filter paper and subsequently condensing excess humidity by leading the air stream through a water bath of known temperature.

3.5.3 Determination of H_2O and CO_2 exchange

A seedling was enclosed in the leaf chamber (Plate 3.1.) and readings of light intensity and temperature, relative humidity, and CO_2 concentration of the air stream after the leaf chamber as well as the ambient CO_2 concentration were recorded after the system had been running for 60 seconds. This had been predetermined as the minimum time for steady CO_2 and relative humidity readings; responses of stomata to changes in vapour pressure deficit in Sitka spruce seedlings have been reported to be evident within 4–5 minutes (Watts & Neilson 1978).

3.5.4 Calculations

Transpiration rates, leaf temperatures, net photosynthetic rates and stomatal conductances were computed as directed by the manufacturer, using the boundary layer resistance of 0.314 m^2 s mol^{-1} (determined by C.G.M^{c}K. Henderson and A.P. Sandford). The moisture content of the air entering the leaf chamber which had passed through silica gel was taken into account in the calculations.

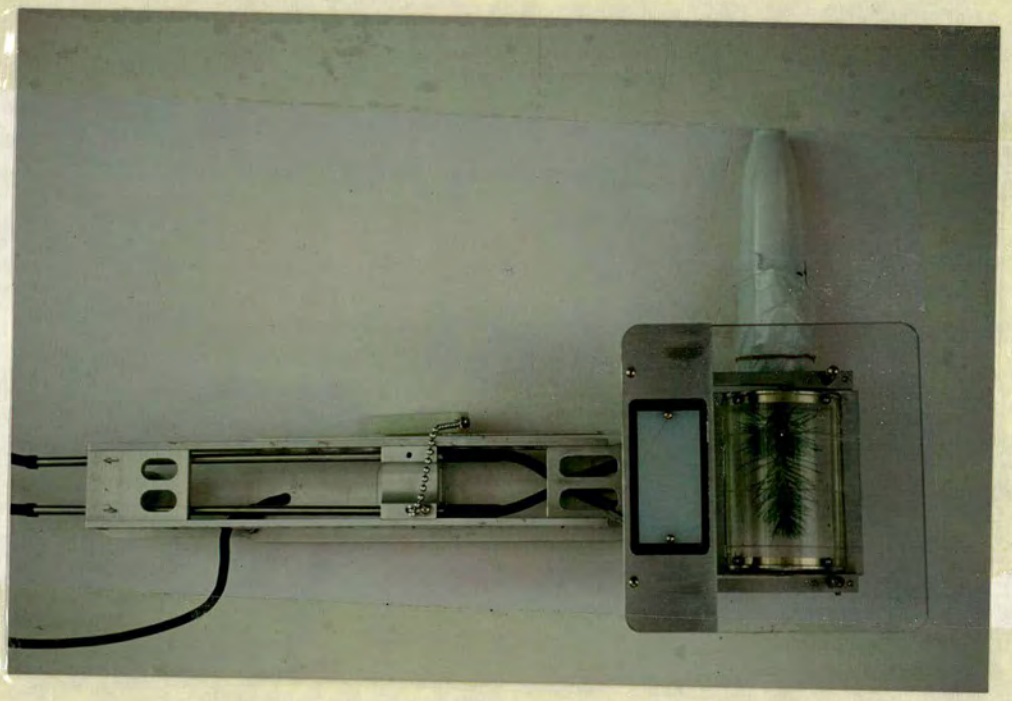


Plate 3.1. A Sitka spruce seedling in a leaf chamber (ADC, PLC2).

3.6 Plant water potential

The shoot water potential of seedlings was measured with a pressure chamber (Department of Forestry and Natural Resources, University of Edinburgh, Edinburgh) immediately after severing the shoot at root collar with a razor blade. The precautions discussed by Ritchie & Hinckley (1975) were taken into account: the amount of stem protruding from the chamber was always less than 1.5 cm; the measurements were finished within 5 min from cutting the stem, normally sooner than that; pressure was applied to the chamber at a rate of 0.03 MPa s^{-1} or less.

The endpoint was usually distinct even though some fluid from intercellular spaces frequently appeared on the cut surface at lower pressures. The amounts were small enough not to interfere with observations.

3.7 Nutrient analyses of plant material

Complete shoots or needle samples were oven-dried at 85°C for 72 h and cooled in a desiccator. Either a complete shoot or a subsample of about 100 mg of needles was weighed and organic matter was digested by incubating with 2 ml H_2SO_4 and 1 ml H_2O_2 in 350°C for 6 h. Samples were made up to 50 ml with water.

Standard solutions with the same H_2SO_4 concentration as sample solutions were prepared with KCl, NH_4Cl and KH_2PO_4, with the following concentrations:
N 0, 5, 10, 15, and 25 mg l^{-1}
P 0.0, 0.5, 1.0, 1.5, and 2.0 mg l^{-1}
K 0, 10, 20, and 30 mg l^{-1}.

Potassium concentrations of the solutions were determined with a Pye Unicam SP9 atomic absorption spectrophotometer (Pye Unicam, Cambridge) used as a flame emission spectrophotometer. Nitrogen and phosphorus concentrations of the same solutions were determined with flow injection analysis using a Tecator 5023 Fia Star spectrophotometer (Tecator, Bristol) connected with a Tecator Fia Star 5020 analyzer and 5007 sampler. Phosphorus was determined with the molybdenum blue method using stannous chloride as reducing agent,

and nitrogen was determined by mixing the sample stream with sodium hydroxide, and mixing the ammonia gas formed with indicator solution (Ammonia indicator mixture, Tecator).

3.8 Statistics and computer programs

The main experiments were designed as completely randomized factorial experiments with or without blocks and analyzed accordingly with one-way or two-way analysis of variance. The suitability of data for analysis of variance (fulfilment of the assumptions of normality of distribution, homogeneity of variance, and additivity of treatment effects) was confirmed by examination of the data, and if necessary, appropriate transformations were used. In the cases of proportions (mycorrhizal percentages, root / shoot ratios, root tip number / root dry weight ratios, and water use efficiency (transpiration rate / net photosynthetic rate)) the angular transformation was used to normalize distributions in all statistics except computing means and their standard errors. Tukey's test was used to test differences between means when variance ratios indicated significance.

Genstat language (Lawes Agricultural Trust, Rothamsted) was used for writing programs for analysis of variance and covariate analysis, and correlation and regression analyses. In regression analysis, Genstat uses an 'adjusted R square' which is calculated using mean squares rather than sums of squares. This figure was used as the estimate for the percentage variance accounted for by models.

The graphs were produced using the Easygraph program provided by Edinburgh Regional Computing Centre.

CHAPTER 4
PHOTOSYNTHESIS AND WATER RELATIONS OF ECTOMYCORRHIZAL AND NONMYCORRHIZAL PLANTS IN CONDITIONS OF LOW NUTRITION

4.1 Introduction

The results from experiments comparing water relations of ectomycorrhizal and nonmycorrhizal plants have been somewhat conflincting (Section 2.3.5.). Sometimes mycorrhizal seedlings have taken up water more efficiently during drought compared to nonmycorrhizal (Dixon *et al.* 1980, 1983, Boyd 1987, Osonubi 1989), but experiments on radiata pine (Sands & Theodorou 1978) and Douglas fir (Parke *et al.* 1983) in relation to their resistance to suddenly imposed drought have not shown any definite advantage from mycorrhizal structure as opposed to nonmycorrhizal root systems. As the results obtained so far are by no means exhaustive, an experiment was set up to study whether mycorrhizal Sitka spruce seedlings have better resistance to a drying cycle than nonmycorrhizal, as indicated by gas exchange, water potential, and growth characteristics. *Paxillus involutus* was chosen as the test fungus because of its widespread occurrence in habitats liable to drying out.

4.2 Materials and methods

Two experiments were conducted in parallel, 1a in which gas exchange and shoot water potentials of the plants were measured at different stages of the drying and rewatering cycle, and 1b where transpiration was measured gravimetrically throughout the cycle.

Sitka spruce seedlings were grown in vermiculite-peat in 170 cm^3 'Ray Leach' tubes (Plate 4.1.) in a glasshouse with minimum temperature of 20°C and with daylight being supplemented by high pressure mercury lamps (400 W MBFR/U) to extend the daylength to 16 h (Section 3.1.). Twenty-four days after germination half of the plants were inoculated with a liquid culture of *Paxillus involutus* and half were left uninoculated. Twenty-one days later the inoculated plants were reinoculated. Seventy-five days after germination the

plants were transferred to a walk-in growth room to acclimatize at 18°C day and night temperature, photoperiod 16 h, illumination with cool white very high output fluorescent tubes (Sylvania) approximately 380 µmol $m^{-2}s^{-1}$ PAR, and relative humidity 75 % corresponding to 0.52 kPa vapour pressure deficit for 16 days before the beginning of the water stress treatment. By this time, each plant had been fertilized with Ingestad solution corresponding to a total of 1.7 mg N. This was given in several small doses on different days. Plants were completely randomly allocated to different treatments and harvests in Experiments 1a and 1b.

Experiment 1a. The experimental design was a completely randomized 2 x 2 factorial with two fungal treatments (mycorrhizal and nonmycorrhizal) and two water regimes (well-watered and unwatered). Three sets of measurements were performed:
(1) before droughting (gas exchange measurements and harvest 1 on days 1-3),
(2) at peak of the drought (last watering on day 0, gas exchange measurements and harvest 2 on days 26-27),
(3) and after a period of recovery (rewatering on day 27, gas exchange measurements and harvest 3 on days 36-37).

The measurement of CO_2 and H_2O exchange of groups of 4 plants was carried out in light (480 µmol $m^{-2}s^{-1}$) and dark as described in Section 3.4. The measurements started 6 h after the onset of the light period and went on until dark.

Hence the plants were 128 days old at the end of the experiment. While other plants were subjected to drought, the well-watered treatment was watered with 5 ml distilled water daily, which was approximately the amount of water loss from the pots, determined in preliminary experiments. The daily water loss by transpiration from each pot was about one-tenth of the evaporation, and relative to this, the differences between the amount of water transpired by the large mycorrhizal plants and the smaller nonmycorrhizal plants were considered negligible. Therefore the amount of water added daily to all plants in the well-watered treatment was the same. The plants in set 3 were rewatered with water and an Ingestad solution corresponding to a total of 0.7 mg N for each plant.

After gas exchange measurements the seedlings were removed to a cold room

(+3°C), except those randomly chosen ones whose shoot water potentials were to be measured at harvests 2 and 3. The water potential measurements made use of a pressure chamber, and they were completed within a 4 h period beginning 11 h after the onset of the light period on the same day as the gas exchange of the plants had been measured. At harvest 2, the moisture content of the substrate around the lowest part of the root system (Fig. 4.1.) was determined gravimetrically.

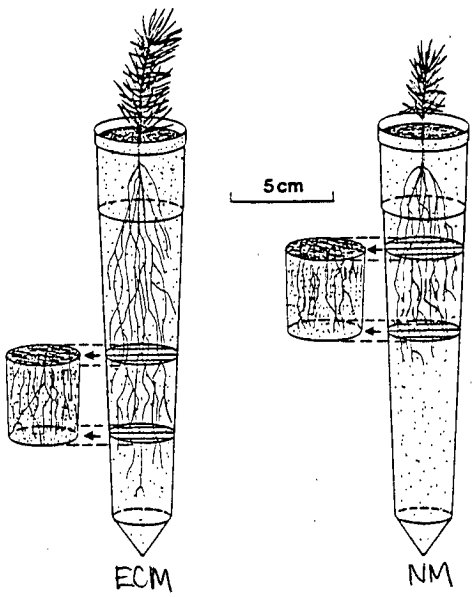


Fig. 4.1. Location of substrate sampled for determination of moisture content around root systems of mycorrhizal (ECM) and nonmycorrhizal (NM) Sitka spruce seedlings at harvest 2 in Exp. 1a (see text for harvests).

At each harvest the plants were removed from the vermiculite-peat medium by washing, and the following measurements made:
length of three longest long roots,
root tip numbers and mycorrhizal root tip numbers,
root and shoot dry weight,
and shoot N, P and K concentrations (Chapter 3).

Needle area was determined using a Delta-T area meter (Delta-T devices, Cambridge, England). Readings from the meter were transformed to surface areas by measuring sets of pieces of plastic coated wire. Wire of known width

was cut in lengths to simulate needles, and a calibration line was drawn to relate the area of the wire to meter readings.

At harvest 1 the drought treatment had not yet started, and there were 8 replicate groups of 4 plants for each fungal treatment. At harvests 2 and 3 there were 4 replicate groups of 4 plants for each of the 4 combinations of treatments.

The data were subjected to analysis of variance, and if the variance ratios indicated significance for the interaction of watering and inoculation treatments, least significant differences ($P<0.05$) between individual treatment means were calculated using Tukey's test. Otherwise only significances of the the main treatment differences are reported at 0.05 and 0.01 levels.

The angular transformation was used for proportions. Because of the large difference between the mycorrhizal and nonmycorrhizal plants, in the case of some variables (dry weight, root tip numbers, leaf area, P concentration, N, P and K contents) the variances were larger for the mycorrhizal treatment than nonmycorrhizal. Therefore the analysis of variance was repeated using a natural logarithm transformation of these variables, and when this yielded different significance levels from the analysis with untransformed data, the analysis with logarithms is reported. This also took account of the possibility that the differences between the fungal treatments in their relation to different water regimes might have been proportional rather than additive.

Experiment 1b. A separate set of 10 plants per treatment was grown and treated in the same way as plants in Experiment 1a and used for gravimetric measurements of transpiration rates every two or three days throughout the experiment (Section 3.3.). The measurements were done at the same time of the day (±1 h) beginning 9 h after the onset of the light period. When the pots were being sealed and weighed, they were outside the growth room for approximately 30 min.

The heights of this set of plants were measured four times in the course of the experiment. The heights were measured with a ruler from the bottom of the lowest needles to the tips of the top needles. The leaf areas were measured at the end of the experiment. Relative height growth rates over the 30 days between the beginning of the drought treatment (height = h1) and four days after rewatering (h2) were calculated as:

$$\frac{\log(h2) - \log(h1)}{30}$$

A natural logarithm transformation was used in the analysis of variance of the whole-plant transpiration for the same reason as in Experiment 1a.

4.3 Results

4.3.1 Mycorrhizal formation and growth of plants

Some of the replicate plants were contaminated by airborne spores of mycorrhizal fungi, usually *Thelephora terrestris*. With the obvious exception of analysis of proportions of mycorrhizas formed by contaminating fungi, these plants were discarded from the analysis. The number of replicates remaining for each treatment is shown in Table 4.1., and the original treatment means are shown in Tables 4.1.a-4.4.a in Appendix A.

The amount of contamination increased towards the end of the experiment (Table 4.1.); in the first two harvests, before and during drought, numbers of *Thelephora* were small. At harvest 2, there was significantly more *Thelephora* in the watered plants than droughted (Table 4.1.). At harvest 3, the mean of *Thelephora* mycorrhizal root tips was not significantly different in the different treatments. Because of the small number of replicates at harvest 3, the analysis of variance of the growth and nutrition of these plants (Tables 4.1.-4.4.) must only be taken as suggestive of treatment differences.

The proportions of *Paxillus involutus* mycorrhizas increased towards the end of the experiment from the initial 19 % to about 40 %. Droughting did not significantly affect the proportion of *Paxillus* mycorrhizas. The mycorrhizal seedlings were much larger than the nonmycorrhizal (Plate 4.1.), and the inoculation treatment affected the growth of plants more than drought did. The total dry weights (Fig. 4.2.), root tip numbers (Table 4.1.), leaf areas (Table 4.2.), and mean length of three longest roots (Table 4.2.) were all significantly higher in mycorrhizal plants than nonmycorrhizal at each harvest. Root / shoot ratios were always significantly higher in nonmycorrhizal plants.

Table 4.1. Numbers of root tips and mycorrhizal percentage of Sitka spruce seedlings either inoculated with *Paxillus involutus* (ECM) or noninoculated (NM), ± s.e. Harvest 1: 9 weeks after inoculation, harvest 2: 12 weeks after inoculation, w = well-watered, d = not watered for 3 weeks, harvest 3: after 1 week's recovery. * indicates significance at 0.05 level, ** at 0.01 level in analysis of variance. If interaction of mycorrhizal and watering treatments is significant, differences between treatment means are indicated with letters (Tukey's test at $p<0.05$).

Harvest 1

	n	Number of root tips	*Paxillus* mycorrhizas %	Other mycorrhizas %
ECM	30	172±11	19±3.8	3.9±0.3
NM	31	121± 6	0±0.0	0.2±0.2
Significance of differences of means				
ECM–NM		**	**	ns

Harvest 2

	n	Number of root tips	*Paxillus* mycorrhizas %	Other mycorrhizas %
ECM w	14	345±30 a	38±6.0	1.4±0.9
ECM d	16	247±25 b	46±8.2	0.0±0.0
NM w	11	160±22 c	0±0.0	5.1±2.2
NM d	16	164±12 c	0±0.0	0.0±0.0
Significance of differences of means and interaction (i.a.)				
ECM–NM		**	**	ns
w – d		ns	ns	**
i.a.		*	ns	ns

Harvest 3

	n	Number of root tips	*Paxillus* mycorrhizas %	Other mycorrhizas %
ECM w	8	444±46	38±8.0	12.2±4.8
ECM d	13	438±38	43±8.1	9.9±6.1
NM w	9	286±29	0±0.0	12.2±5.7
NM d	5	215±26	0±0.0	27.4±6.7
Significance of differences of means and interaction (i.a.)				
ECM–NM		**	**	ns
w – d		ns	ns	ns
i.a.		ns	ns	ns

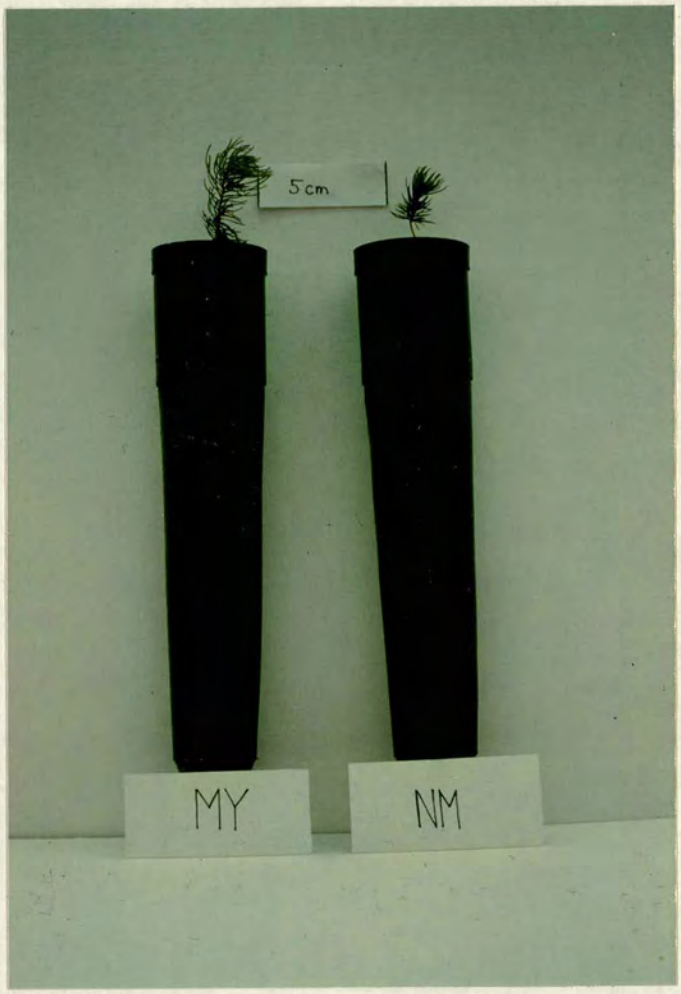


Plate 4.1. A mycorrhizal (MY, *Paxillus involutus)* and a nonmycorrhizal (NM) Sitka spruce seedling at the end of Exp. 1.

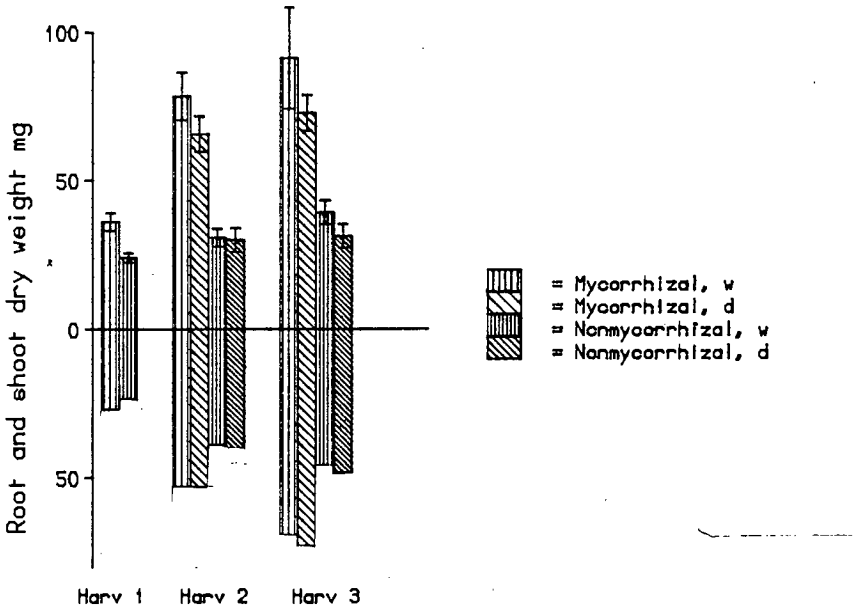


Fig. 4.2. Dry weights of Sitka spruce seedlings mycorrhizal with *Paxillus involutus* or nonmycorrhizal, before (harvest 1), during (harvest 2) and after (harvest 3) a drying cycle. w = well-watered, d = droughted. Means ± s.e., replicate numbers as in Table 4.1. Main effect of inoculation significant ($p<0.01$) in each harvest, effect of water regime and interactions nonsignificant in analysis of variance.

The droughting reduced the dry weight increment of the mycorrhizal plants slightly but not significantly relative to controls by the time of harvest 2. The only statistically significant effects of drought on plant growth by harvest 2 were on the number of root tips in the mycorrhizal treatment, which was lower in unwatered plants both as absolute numbers (log transformed) (Table 4.1.) and per unit root dry weight (Table 4.2). Root tip number was no longer different in the two water regimes at harvest 3, but by then the leaf area was smaller in both mycorrhizal and nonmycorrhizal drought treated plants. Shoot dry weight behaved in the same way as leaf area (Fig. 4.2., ANOVA not shown). Even though the root / shoot ratio was not higher in plants of the dry treatment at harvest 2, at harvest 3 it was. The growth of nonmycorrhizal plants during the experiment was slow, but mycorrhizal plants grew at a slightly higher rate (Fig. 4.2).

Table 4.2. Characteristics of *Paxillus involutus* inoculated (ECM) and noninoculated (NM) Sitka spruce seedlings before (harvest 1), during (harvest 2) and after (harvest 3) exposure to drought ± s.e. w = well-watered, d = droughted. * indicates significance at 0.05 level, ** at 0.01 level in analysis of variance. If interaction of mycorrhizal and watering treatments is significant, differences between treatment means are indicated with letters (Tukey's test at $p<0.05$).

Harvest 1

	Leaf area cm^2	Root / shoot ratio	Mean of 3 longest roots mm	No. tips / mg dwt
ECM	2.9±0.20	0.69±0.03	146± 6	6.4±0.33
NM	1.7±0.05	0.99±0.04	123± 4	5.2±0.19
Significance of differences of means				
ECM–NM	**	**	**	**

Harvest 2

	Leaf area cm^2	Root / shoot ratio	Mean of 3 longest roots mm	No. tips / mg dwt
ECM w	6.2±0.35	0.69±0.05	175±9	6.6±0.54 a
ECM d	5.4±0.47	0.85±0.05	176±6	4.6±0.43 b
NM w	2.0±0.14	1.41±0.20	126±6	4.3±0.53 b
NM d	1.9±0.10	1.31±0.07	135±5	4.2±0.27 b
Significance of differences of means and interaction (i.a.)				
ECM–NM	**	**	**	**
w - d	ns	ns	ns	*
i.a.	ns	ns	ns	*

Harvest 3

	Leaf area cm^2	Root / shoot ratio	Mean of 3 longest roots mm	No. tips / mg dwt
ECM w	7.1±0.78	0.84±0.07	184±12	6.6±0.43
ECM d	5.6±0.31	1.06±0.08	192± 5	6.0±0.41
NM w	2.7±0.22	1.15±0.04	151± 6	6.5±0.57
NM d	2.0±0.15	1.59±0.16	150± 9	4.5±0.49
Significance of differences of means and interaction (i.a.)				
ECM–NM	**	**	**	*
w - d	**	**	ns	**
i.a.	ns	ns	ns	ns

4.3.2 Nutrition

The overall levels of nitrogen, phosphorus and potassium were low (Benzian & Smith 1973), especially in nonmycorrhizal plants (Table 4.3); the total P content of shoots of nonmycorrhizal plants was only about one-tenth of that of mycorrhizal ones (Table 4.4.). The P content of inoculated plants at harvest 1 was not related to the mycorrhizal proportion, but all the plants within the inoculated treatment contained more P than nonmycorrhizal plants (Fig. 4.3.). At the first harvest, the mean N concentration was the same in the mycorrhizal and nonmycorrhizal treatment, but later the mycorrhizal plants had a significantly lower concentration than nonmycorrhizal. However, the amount of N in the shoot was significantly higher in mycorrhizal plants. P (log transformed) and K concentrations were considerably higher in mycorrhizal plants, decreasing from harvest 1 to harvest 2. At harvest 3, after rewatering with nutrient solution, percentages of all nutrients had recovered slightly.

Droughting had a significant effect on the N concentration of nonmycorrhizal plants, but not mycorrhizal, the N level being lower in unwatered plants. At harvest 3, this effect was reversed, and the water stressed nonmycorrhizal plants had higher N percentages than the well-watered ones. The K concentrations of droughted plants were significantly lower in both harvest 2 and 3.

All total nutrient contents were significantly lower in shoots of nonmycorrhizal plants. The drying treatment led to lower N, P and K contents of plants in harvest 2, combining the drought effect on shoot growth and nutrient concentration. By harvest 3 the drought effects on nutrient contents had disappeared for N and P, but not K.

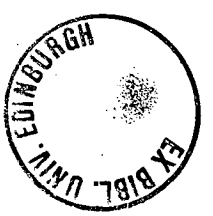


Table 4.3. N, P and K concentrations of shoots of *Paxillus involutus* inoculated (ECM) and noninoculated (NM) Sitka spruce seedlings before (harvest 1), during (harvest 2) and after (harvest 3) exposure to drought ± s.e. w = well-watered, d = droughted. * indicates significance at 0.05 level, ** at 0.01 level in analysis of variance.

Harvest 1

	N %	P %	K %
ECM	1.87±0.071	0.349±0.010	1.54±0.060
NM	1.80±0.059	0.075±0.011	1.17±0.046
Significance of differences of means			
ECM-NM	ns	**	**

Harvest 2

	N %	P %	K %
ECM w	1.12±0.074	0.253±0.018	1.29±0.067
ECM d	1.12±0.075	0.215±0.009	1.14±0.066
NM w	1.62±0.059	0.053±0.009	0.93±0.046
NM d	1.33±0.040	0.050±0.008	0.83±0.067
Significance of differences of means and interaction (i.a.)			
ECM-NM	**	**	**
w - d	*	ns	*
i.a.	ns	ns	ns

Harvest 3

	N %	P %	K %
ECM w	1.02±0.120	0.234±0.056	1.34±0.162
ECM d	1.22±0.088	0.289±0.022	1.20±0.068
NM w	1.43±0.086	0.040±0.011	1.13±0.071
NM d	1.61±0.110	0.050±0.011	0.87±0.106
Significance of differences of means and interaction (i.a.)			
ECM-NM	**	**	**
w - d	*	*	*
i.a.	ns	ns	ns

Table 4.4. N, P and K contents of shoots of *Paxillus involutus* inoculated (ECM) and noninoculated (NM) Sitka spruce seedlings before (harvest 1), during (harvest 2) and after (harvest 3) exposure to drought ± s.e. w = well-watered, d = droughted. * indicates significance at 0.05 level, ** at 0.01 level in analysis of variance.

Harvest 1

	N mg	P mg	K mg
ECM	0.65±0.031	0.124±0.007	0.57±0.044
NM	0.43±0.018	0.018±0.003	0.28±0.012
Significance of differences of means			
ECM-NM	**	**	**

Harvest 2

	N mg	P mg	K mg
ECM w	0.84±0.044	0.188±0.009	0.99±0.075
ECM d	0.71±0.047	0.139±0.008	0.75±0.071
NM w	0.50±0.035	0.016±0.003	0.28±0.025
NM d	0.40±0.019	0.015±0.002	0.24±0.020
Significance of differences of means and interaction (i.a.)			
ECM-NM	**	**	**
w - d	*	ns	*
i.a.	ns	ns	ns

Harvest 3

	N mg	P mg	K mg
ECM w	0.88±0.082	0.191±0.030	1.16±0.099
ECM d	0.86±0.043	0.206±0.017	0.87±0.075
NM w	0.55±0.035	0.018±0.006	0.45±0.044
NM d	0.49±0.026	0.016±0.004	0.28±0.049
Significance of differences of means and interaction (i.a.)			
ECM-NM	**	**	**
w - d	ns	ns	**
i.a.	ns	ns	ns

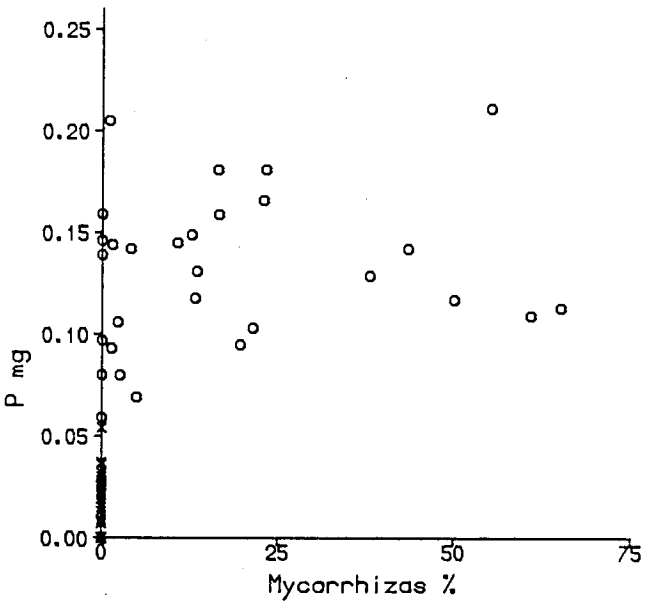


Fig. 4.3. Shoot phosphorus content of Sitka spruce seedlings in relation to mycorrhizal *(Paxillus involutus)* proportion in Exp. 1a, harvest 1. Circle, inoculated; cross, noninoculated.

4.3.3 Plant and soil water status

The shoot water potential was higher in the well-watered mycorrhizal plants than well-watered nonmycorrhizal in harvest 2 (Table 4.5.). At the peak of the drought the mean water potential of nonmycorrhizal plants was the same in the watered and droughted treatment, and although the mycorrhizal droughted plants had decreased water potentials, these were similar to the nonmycorrhizal controls. The unwatered mycorrhizal plants had also dried out the substrate more efficiently around the lowest part of their root systems than the nonmycorrhizal plants (a in Table 4.5.). The water content of the whole pot in Experiment 1b is shown in this context for comparison (b in Table 4.5.). This was the same between the unwatered mycorrhizal and nonmycorrhizal plants, but in the well-watered treatment the mycorrhizal plants had dried the substrate out more efficiently, contrary to the assumption made at the onset of the experiments (Section 4.2.). However, the water content in the root environment is a better measure of water availability to the plants, and this was similar for the mycorrhizal and nonmycorrhizal plants in the watered treatment.

Table 4.5. Shoot water potential (ψ) of Sitka spruce seedlings either inoculated with *Paxillus involutus* (ECM) or noninoculated (NM), ± s.e. Harvest 2: 12 weeks after inoculation, w = well-watered, d = not watered for 3 weeks, harvest 3: after 1 week's recovery. For Harvest 2, respective mean of substrate moisture content (% of dry soil) indicated a) in a sample from around lowest roots, b) in the whole pot (Exp. 1b, mean of 10 replicates), ± s.e.

Harvest 2

	n	ψ MPa	Soil moisture content % a)	Soil moisture content % b)
ECM w	3	-0.80±0.05	363±26	365±5
ECM d	4	-1.07±0.17	161±10	168±6
NM w	4	-1.11±0.03	367±16	399±5
NM d	3	-1.12±0.02	277±43	169±6

Harvest 3

	n	ψ MPa
ECM	3	-0.85±0.09
NM	5	-1.03±0.04

Because the replicate numbers were low after the removal of contaminated seedlings and because there was apparently no difference between the watered and dry treatment, the water potential measurements of watered and droughted plants were combined at harvest 3. The difference between mycorrhizal and nonmycorrhizal plants was similar to the difference between the watered control plants at harvest 2.

4.3.4 Gas exchange

The relationship between light intensity and net photosynthetic rate measured on two plants in preliminary experiments is shown in Fig. 4.4. At 480 µmol $m^{-2}s^{-1}$ net photosynthesis appears to be near light saturation.

At the first harvest, there was a significant difference between the net photosynthetic rates per unit leaf area of mycorrhizal and nonmycorrhizal plants (Table 4.6.), which was probably mainly due to the different nutrient status of the plants. The relationship between shoot P concentration and photosynthesis at harvest 1 is shown in Fig. 4.5.

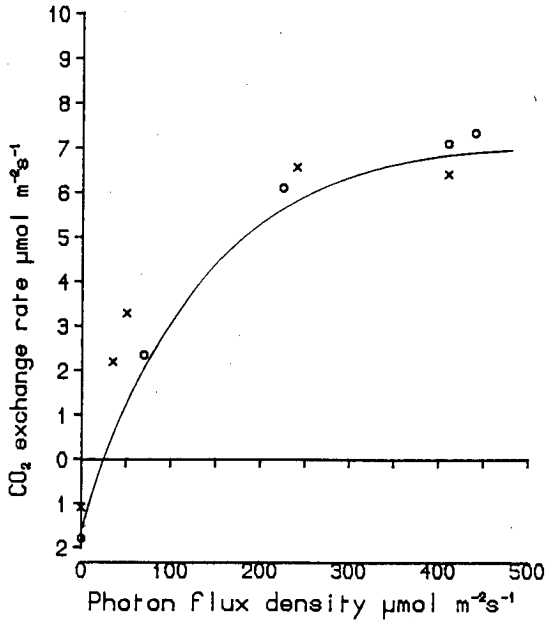


Fig. 4.4. Light response of net photosynthetic rates of two sets of four Sitka spruce seedlings. Line fitted by the eye.

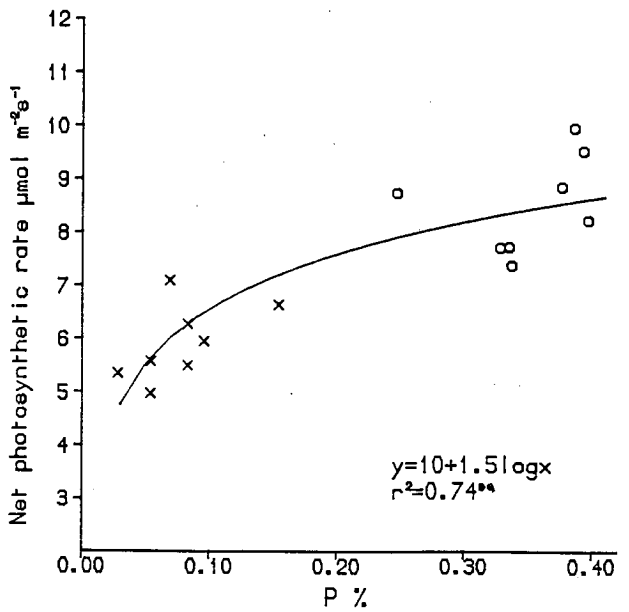


Fig. 4.5. Relationship of shoot phosphorus concentration and net photosynthetic rate of mycorrhizal (circle) and nonmycorrhizal (cross) 3-month-old Sitka spruce seedlings in Exp. 1a at harvest 1. Photosynthesis measured on sets of four plants, P % is mean of four plants.

Table 4.6. Stomatal conductance to water vapour, CO_2 exchange, and water use efficiency (WUE) of Sitka spruce seedlings either inoculated with *Paxillus involutus* (ECM) or noninoculated (NM), ± s.e. Harvest 1: 9 weeks after inoculation, harvest 2: 12 weeks after inoculation, w = well-watered, d = not watered for 3 weeks, harvest 3: after 1 week's recovery. * indicates significance at 0.05 level, ** at 0.01 level in analysis of variance. If interaction of fungal and watering treatments is significant, differences of means are indicated with letters (Tukey's test at $p<0.05$).

Harvest 1

	Stomatal conductance mmol $m^{-2}s^{-1}$	Net photo-synthesis µmol $m^{-2}s^{-1}$	Dark respiration µmol $m^{-2}s^{-1}$	WUE µmol CO_2 / mmol H_2O
ECM	298±35	8.5±0.33	1.41±.045	7.7±0.42
NM	158±14	5.9±0.25	1.22±.054	6.0±0.42
Significance of differences of means				
ECM-NM	**	**	*	*

Harvest 2

	Stomatal conductance mmol $m^{-2}s^{-1}$	Net photo-synthesis µmol $m^{-2}s^{-1}$	Dark respiration µmol $m^{-2}s^{-1}$	WUE µmol CO_2 / mmol H_2O
ECM w	187±22	4.9±0.26	0.77±.046	6.1±0.35
ECM d	80± 6	3.7±0.23	0.63±.070	7.8±0.29
NM w	150±19	4.5±0.36	0.47±.073	5.1±0.68
NM d	79± 3	3.0±0.40	0.68±.110	5.2±0.79
Significance of differences of means and interaction (i.a.)				
ECM-NM	ns	ns	ns	**
w - d	**	**	ns	ns
i.a.	ns	ns	*	ns

Harvest 3

	Stomatal conductance mmol $m^{-2}s^{-1}$	Net photo-synthesis µmol $m^{-2}s^{-1}$	Dark respiration µmol $m^{-2}s^{-1}$	WUE µmol CO_2 / mmol H_2O
ECM w	198±17	5.3±0.26 a	0.62±.094	6.1±0.40
ECM d	179±12	5.1±0.04 a	0.55±.078	6.0±0.26
NM w	135±18	3.9±0.06 b	0.38±.084	4.8±0.54
NM d	164± 8	5.6±0.22 a	0.39±.099	5.5±0.29
Significance of differences of means and interaction (i.a.)				
ECM-NM	*	*	*	*
w - d	ns	**	ns	ns
i.a.	ns	**	ns	ns

By the time of the second harvest, the overall level of photosynthesis had decreased, and there was only a slight, nonsignificant difference between mycorrhizal and nonmycorrhizal plants.

Drought significantly decreased net photosynthetic rates of both mycorrhizal (to 76 % of controls) and nonmycorrhizal plants (67 %). After rewatering with nutrient solution, the photosynthesis of the drought treated plants had recovered completely, but the photosynthetic rates of the watered nonmycorrhizal controls were low, and this together with the high rates of the contaminated droughted 'nonmycorrhizal' plants caused a strong interaction.

Dark respiration rates were usually proportional to net photosynthetic rates, and they were significantly higher for the mycorrhizal treatment at the first set of measurements. At the peak of the drought, the respiration rates were decreased in the droughted mycorrhizal treatment relative to their controls, but increased in the nonmycorrhizal treatment.

Stomatal conductances of mycorrhizal plants were significantly higher than those of nonmycorrhizal at the first and third sets of measurements. At the peak of drought, droughting had a significant effect on stomatal conductance, which was not significantly different in the two inoculation treatments. The relation of stomatal conductance and net photosynthetic rate at harvest 2 is shown in Fig. 4.6. This was different for mycorrhizal and nonmycorrhizal plants, mycorrhizal having somewhat higher CO_2 uptake rate at the same stomatal conductance than nonmycorrhizal.

Water use efficiency calculated as (net photosynthetic rate of 4 plants) / (transpiration rate of 4 plants) was significantly higher in mycorrhizal plants than nonmycorrhizal at harvests 1 and 2, and slightly but not significantly increased during drought in the mycorrhizal treatment.

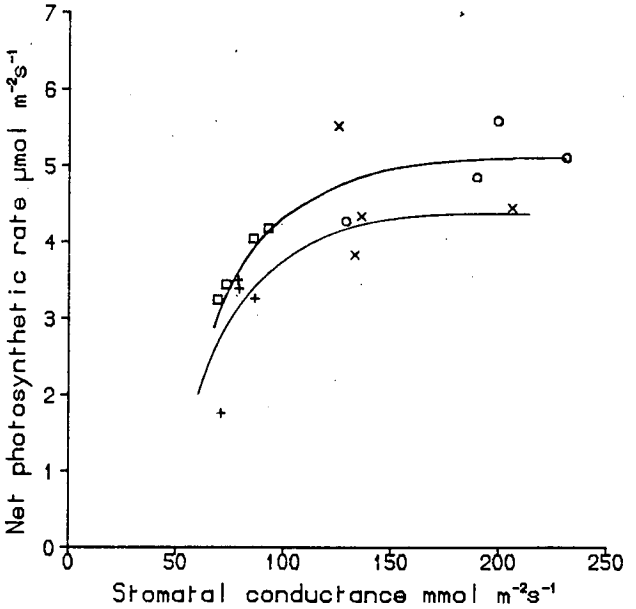


Fig. 4.6. Relationship of stomatal conductance and net photosynthetic rate in sets of four Sitka spruce seedlings in Exp. 1 at harvest 2. Circle, mycorrhizal *(Paxillus involutus)* well-watered; square, mycorrhizal droughted; cross, nonmycorrhizal well-watered; plus, nonmycorrhizal droughted. Lines fitted by the eye.

4.3.5 Growth and transpiration of plants in Experiment 1b

The height growth of plants in Experiment 1b is shown in Fig. 4.7. The main treatment effect of inoculation was highly significant ($P<0.01$) throughout, and at the two last measurements, four days after rewatering and after the recovery period, the water stressed plants were significantly shorter than well-watered. This effect was mainly due to the check in the growth of mycorrhizal plants, and at the end of the experiment there was a significant interaction of watering and inoculation, as mycorrhizal plants were more affected by drought than nonmycorrhizal. This is reflected in the relative height growth rates as well. Between the beginning of the experiment (day 1) and four days after rewatering (day 31) these were ($\mu m\ mm^{-1}d^{-1}$):

ECM watered	8.4 a
ECM droughted	3.9 b
NM watered	8.0 a
NM droughted	8.0 a

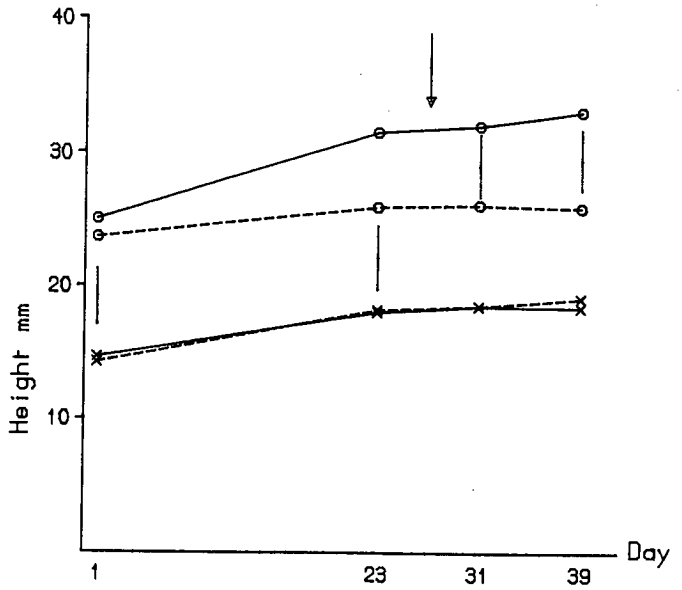


Fig. 4.7. Height growth of Sitka spruce seedlings during Exp. 1b. Circle, mycorrhizal; cross, nonmycorrhizal. Solid line, well-watered; dashed line, drought treated. Drought treatment commenced on day 1, arrow shows time of rewatering. Means of 10 replicate plants. Bars indicate least significant difference between individual means ($p<0.05$, Tukey's test on untransformed data).

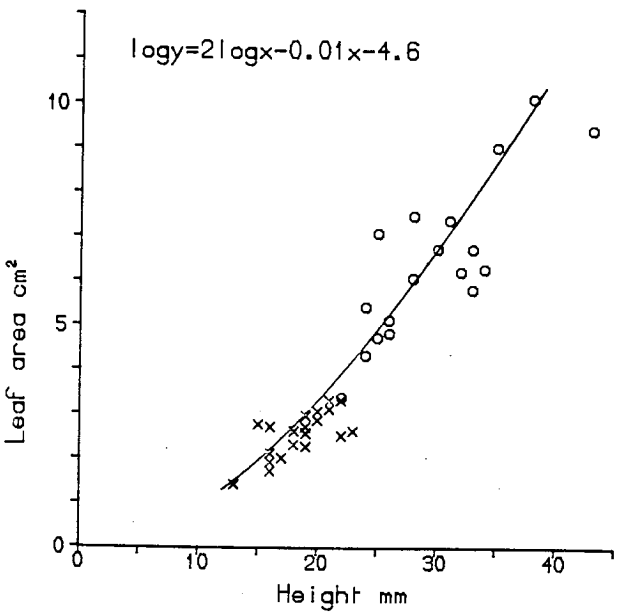


Fig. 4.8. Relationship of height and leaf area of 4-month-old Sitka spruce seedlings at the end of Exp. 1b. Circle, mycorrhizal *(Paxillus involutus)*; cross, nonmycorrhizal.

The relationship between leaf area and height of the plants at the end of the experiment was used to estimate the leaf areas at other times. The regression model used was chosen as the one which yielded transpiration rates closest to those computed using measured leaf areas for the last day before the harvest, day 38 (Fig. 4.8.). The means of calculated and measured leaf areas and transpiration rates were:

	Leaf area cm^2		Transpiration mmol $m^{-2}s^{-1}$	
	Measured	Calculated	Measured	Calculated
ECM, w	7.8	7.9	0.89	0.94
ECM, d	5.1	5.6	0.91	0.85
NM, w	2.4	2.8	0.94	0.80
NM, d	2.6	2.9	0.89	0.80

Hence the calculated values for small leaf areas were overestimated, and, consequently, the transpiration rates of the smaller nonmycorrhizal and water-stressed mycorrhizal seedlings slightly underestimated. The significance of the differences between treatments was the same for measured and calculated values. The day to day variation in the transpiration rates (Fig. 4.9. and Fig. 4.10.) was probably mostly due to the fact that the plants were out of the growth room before and during the measurements, and the outdoors temperatures varied between about 16°C and 28°C during the experiments.

Within the limits of experimental error and error in estimating the leaf areas of the plants at different times, the initial transpiration rates of mycorrhizal and nonmycorrhizal plants were not significantly different (Fig. 4.9.), even though the initial transpiration rate of the dry mycorrhizal treatment was low compared to other treatments. It took 18 days before there was a significant difference between the transpiration rates of the well-watered and droughted treatments. At the peak of drought mycorrhizal and nonmycorrhizal plants did not differ from each other, and they recovered their transpiration rates in a similar way. The difference between watered and dried plants was still significant 5 days after rewatering, however, this may be partly due to the overestimated transpiration rates of mycorrhizal, well-watered plants.

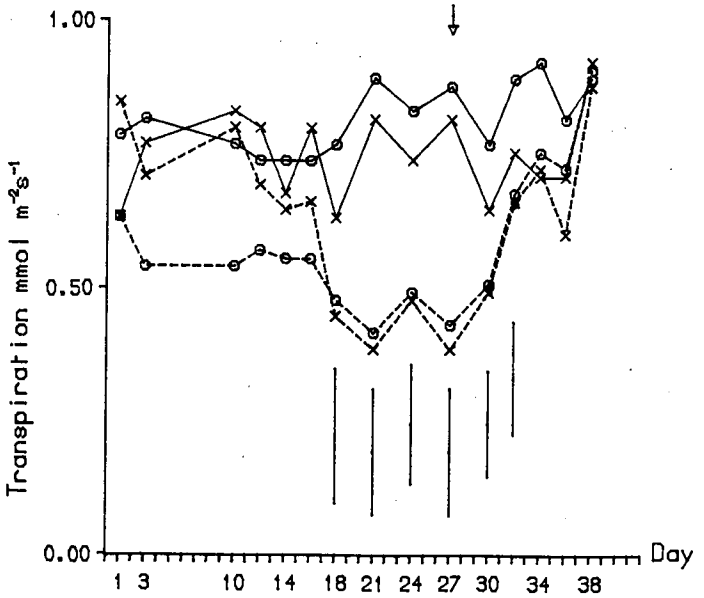


Fig. 4.9. Transpiration rate per unit leaf area of Sitka spruce seedlings in Exp. 1b. Symbols as in Fig. 4.7. Leaf area calculated as described in text except for day 38. Means of 10 plants (except day 1, n=5), bars indicate least significant difference between individual means ($p<0.05$, Tukey's test) where applicable.

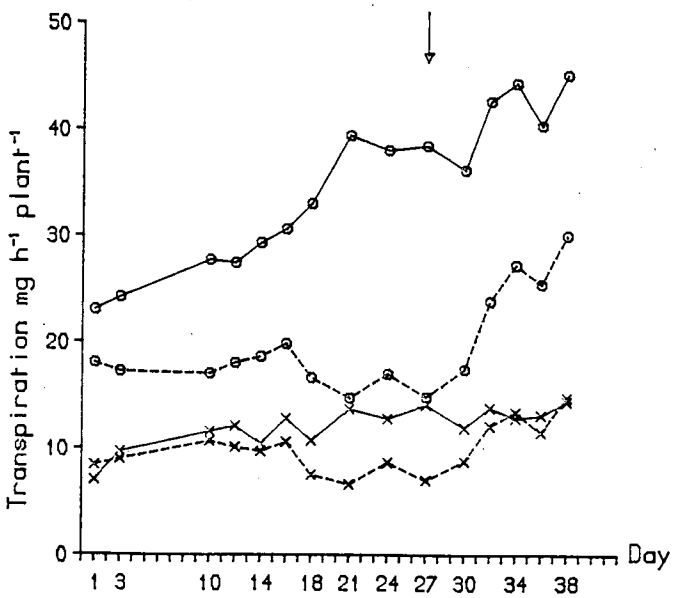


Fig. 4.10. Transpiration of whole Sitka spruce seedlings in Exp. 1b. Symbols as in Fig. 4.7. Means of 10 plants (except day 1, n=5). Analysis of variance on log transformed data indicated significance of the inoculation treatment on each day ($p<0.01$). The difference between the watering treatments was significant from day 21 to day 32 ($p<0.01$), and from day 34 to day 38 ($p<0.05$).

Transpiration of whole plants is not subject to the sources of error involved in calculation of transpiration per unit leaf area. Fig. 4.10. shows that the water loss from the substrate by transpiration at the peak of drought did not differ for the unwatered mycorrhizal plants and the watered nonmycorrhizal plants, in spite of the much larger leaf area in the mycorrrhizal treatment. The difference between the watering treatments was significant from day 21 onwards. On day 27, the ratio between the transpiration of droughted and well-watered plants was 0.39 for the mycorrhizal treatment and 0.50 for the nonmycorrhizal treatment. At this stage the size of the stressed mycorrhizal plants was more affected than that of the nonmycorrhizal plants however, and the corresponding ratio for height was 0.82 for mycorrhizal plants and 1.00 for nonmycorrhizal plants.

4.4 Discussion

4.4.1 Mycorrhizas, nutrition and growth of plants

Mycorrhizal infection had a very pronounced effect on plant growth, even though the average mycorrhizal proportion was initially only 19 %, and always under 50 %. For nonmycorrhizal plants, P was clearly the factor limiting growth, being well below deficiency limits as defined by Binns *et al.* (1980), whilst mycorrhizal plants had normal shoot P concentrations. Nitrogen availability limited the growth of mycorrhizal plants rather than phosphorus in the later harvests, when the N concentrations of the shoots had decreased to values near 1 %, but the N levels of nonmycorrhizal plants were higher due to a concentration effect; they were not able to use the nitrogen because of phosphorus deficiency. Potassium availability might not have been expected to be a factor limiting growth in vermiculite-peat, because vermiculite contains exchangeable K in its lattice structure, but yet mycorrhizal plants were able to take up more K than nonmycorrhizal.

The efficiency of the mycorrhizal root systems is reflected in the lower root / shoot ratios of mycorrhizal plants - they did not invest as much in the root systems as the nutrient stressed nonmycorrhizal plants did. The higher uptake rates of P and K by mycorrhizal plants were at least partly due to their deeper root systems, greater density of root tips and the extensive mycelial

strands which *Paxillus involutus* formed. However, as the plants were larger, they also required more nutrients for their growth, yet the P and K concentrations as well as contents were larger than those of shoots of nonmycorrhizal plants.

Ekwebelam & Reid (1983) also reported P concentrations in mycorrhizal *Pinus contorta* seedlings which were many times higher compared to those of nonmycorrhizal seedlings grown in vermiculite-peat. Their results were obtained as soon as six weeks after inoculation with *Pisolithus tinctorius* and *Suillus granulatus,* which had formed about 5 % mycorrhizas. The increased nutrient uptake led to increased growth and photosynthetic rates of the mycorrhizal plants relative to controls, in the same way as in the experiment described here. Apparently, mycorrhizal effects on phosphorus uptake are not only mediated by an increased surface area of roots and mycelial strands as they begin before the mycorrhizal structure is well established. Possible explanations are the more efficient P absorption by mycorrhizas, and breakdown of organic P compounds (Gianinazzi-Pearson & Gianinazzi 1986).

The water stress treatment tended to decrease the shoot N, P and K concentrations rather than increase them, despite the small decrease in shoot growth. Hence the nutrient uptake and translocation to the shoot was not as efficient in dry conditions as watered. This may have been due to the decrease in the numbers and density of root tips in the case of mycorrhizal plants, but it is also possible that the uptake rates were limited because of the limited mobility of these nutrients in the soil, or the decreased absorbing capacity of the roots. Nutrient uptake of plants has frequently been found to be decreased in dry soil (Section 2.2.2.). In this experiment, the mycorrhizal structure did not seem to be of more advantage in dry soil than in watered conditions in terms of nutrient uptake, as the mycorrhizal uptake of P and K during drought was limited in approximately the same proportion as that by nonmycorrhizal roots. This is in concert with the findings of Reid & Bowen (1979) that excised mycorrhizal roots of *Pinus radiata* did not differ from nonmycorrhizal roots in terms of their phosphorus uptake from solutions with low osmotic potentials. However, the possibility that ectomycorrhizal and nonmycorrhizal roots function in a different way in different soil moisture conditions cannot be excluded. This will be discussed further in subsequent chapters.

Drought suppressed the growth of mycorrhizal plants more than nonmycorrhizal, presumably because they were larger, their growth rates were higher, they dried the soil out more efficiently, and hence they were more susceptible to water stress. Therefore the reactions of the mycorrhizal and nonmycorrhizal plants to the drought treatment cannot very well be compared.

The root tip formation of unwatered mycorrhizal plants was more sensitive to drought than the growth of long roots; whilst root tip numbers decreased, root lengths and dry weights were not affected. However, the root tip numbers recovered completely after rewatering, while leaf area did not increase much between the last two harvests. This indicates a tendency to concentrate the growth in roots even after the actual period of water deficit, which shows also in the increase of root / shoot dry weight ratios during recovery.

Another effect of the drying of the substrate was that on mycorrhizal infection. All contamination that had occurred by the end of the drying cycle was in the well-watered plants. This is not surprising, since this contamination was most probably caused by airborne spores of *Thelephora terrestris,* and fungal spores require water for their germination (Cooke & Al-Hamdani 1986). After rewatering, there was contamination in all treatments.

4.4.2 Physiological effects of drought, mycorrhizas and nutrition

The average net photosynthetic rate of the mycorrhizal treatment at the beginning of the experiment, 8.5 µmol $m^{-2}s^{-1}$, was similar to about 9 µmol $m^{-2}s^{-1}$ measured by Watts & Neilson (1978) on potted Sitka spruce seedlings. and the stomatal conductances were within the range measured by Watts & Neilson. The stomatal conductances decreased in the course of the experiment as did the photosynthetic rates, possibly because of decreasing nutrient concentrations. However, a similar decrease was not observed in the transpiration rates in Experiment 1b.

Mycorrhizal infection strongly influenced photosynthesis as may be expected on basis of the growth effects. This effect is likely to have been partly due to the increased stomatal conductance at harvest 1, but not completely, as the photosynthetic rates of mycorrhizal plants were higher than those of nonmycorrhizal plants with the same stomatal conductance both in watered and droughted plants (harvest 2). This shows in the higher water use

efficiency of the mycorrhizal plants as well. The increased photosynthetic rates and water use efficiencies were at least partly caused by the considerably better P and K nutrition of the mycorrhizal than nonmycorrhizal plants.

Any nutrient deficiency will affect photosynthesis (Nátr 1972), and the close relation between the N status of plants and their photosynthetic rate is well known, N deficiency causing a decrease in both stomatal conductance and chlorophyll content of leaves (Nátr 1972, 1975). The P concentrations of plants have not been found to correlate with photosynthetic rates as clearly as N concentrations do (Syvertsen & Graham 1985, Nátr 1972), and it is difficult to generalize on P effects because the results from different studies have been rather inconclusive (Nátr 1972). However, as P is a constituent of many of the key compounds of the energy metabolism of the plant, severe P deficiency certainly affects photosynthetic reactions. In this experiment, a correlation was found between the P concentrations of the plants and their photosynthetic rates, and as stated earlier, this seemed to be due to increased photosynthetic capacity of the tissues as well as somewhat higher stomatal conductance of the mycorrhizal plants than nonmycorrhizal. Sawada *et al.* (1983) concluded that the reduction of CO_2 assimilation rate of dwarf bean *(Phaseolus vulgaris humilis)* in P deficient conditions was caused by a disturbance in the photosynthetic dark reaction rather than stomatal conductance, but Terry & Ulrich (1973a) found a decrease both in stomatal and mesophyll conductance to CO_2 in deficient sugar beet *(Beta vulgaris)*. K deficiency has been found to reduce the assimilation rates of a range of plant species (Nátr 1972, 1975, Ozbun *et al.* 1965) due to decreased stomatal aperture (Cooper *et al.* 1967, Peaslee & Moss 1968), as well as mesophyll conductance (Terry & Ulrich 1973b).

The influences and interactions of different nutrients on plant water relations are not very well understood, but P and K deficiencies have been shown to decrease transpiration rates, and root hydraulic conductances of plants (see Section 2.2.1.). In this experiment, the increased K and P nutrition was one of the possible reasons for the improved water relations of mycorrhizal plants during drought; their water potentials, stomatal conductances, and photosynthetic rates were not lower than those of nonmycorrhizal plants despite the drier soil they were in, and their dark respiration rates were decreased during drought rather than increased.

Because of the strong influence of mycorrhizal inoculation on the nutrition of plants in this experiment, the differences between plants in the different treatments may as well have been caused by differential nutrition as by direct mycorrhizal effects. This was not fully appreciated by Ekwebelam & Reid (1983) and Reid *et al.* (1983) in reports of mycorrhizal inoculation increasing photosynthetic rates of *Pinus taeda* and *Pinus contorta,* in which there was a substantial increase in P uptake caused by mycorrhiza. This type of experiments clearly demonstrate the importance of ectomycorrhizas in natural conditions where N, P, and K availability usually limit growth, but they do not elucidate, whether mycorrhizal infection as such has an influence on plant water stress resistance or not. Therefore, the initial question cannot be answered, but it must be reformulated to be: is there a mycorrhizal effect on plant water stress resistance in conditions of balanced nutrition?

CHAPTER 5
PHOTOSYNTHESIS AND WATER RELATIONS OF ECTOMYCORRHIZAL AND NONMYCORRHIZAL PLANTS WITH BALANCED NUTRITION

5.1 Introduction

The first experiment (Exp.1, Chapter 4) was carried out in conditions of limited nutrient availability, which led to an advantage for mycorrhizal plants over nonmycorrhizal ones. As the mycorrhizal plants were considerably larger, and their phosphorus and potassium concentrations were higher, the results were rather difficult to interpret, as the better performance of mycorrhizal seedlings may have been a result only of their deeper root systems and better nutrient status. Therefore a trial (Experiment 2) was run at four different nutrient levels applied to plants grown in perlite to find a way of producing mycorrhizal and nonmycorrhizal Sitka spruce seedlings of comparable size and nutrient concentrations. The results are presented in Appendix B. These results then were used to produce plants for Exp. 3, the purpose of which was to compare mycorrhizal and nonmycorrhizal seedlings in terms of their performance in water stress conditions using shoot water potential, stomatal conductance and net photosynthetic rate as indicators of the status of the plants.

5.2 Materials and methods

Sitka spruce seeds were sown in 65 cm^3 polythene pots (Plate 3.1.) in acid washed perlite. The plants were germinated in a glasshouse but after that they were kept in a walk-in growth room throughout the experiment in 18°C day and night temperatures, 16 h photoperiod with 270 µmol $m^{-2}s^{-1}$ PAR provided by metal halide fluorescent lamps (Kolorarc MBIF/H) and tungsten lamps, and 75 % relative humidity corresponding to 0.52 kPa vapour pressure deficit. Half of the plants were inoculated with liquid cultured *Paxillus involutus* about 40 days after germination. Initially the pots were watered with distilled water, then after germination daily with 15 mg N l^{-1} Ingestad solution, and from 105 days after germination until the beginning of the experiments with 50 mg N l^{-1}

Ingestad solution using an excess of nutrient solution to make sure that the concentration of the 'soil' solution was that of the feeding solution after application. Höberg (1989) found that the concentration of the soil solution increased measurably due to evaporation and transpiration during the two-day interval he used between applications of nutrient solution in a same type of a system.

Experiment 3a started 135 days after germination (14 weeks after inoculation). 50+50 randomly chosen mycorrhizal and nonmycorrhizal plants were subjected to drying out by ceasing to water them. The last watering was on day 0, and 5+5 plants were harvested on days 1,3,6,10,11,12,13,14,15,16, and 17. The experiment was terminated when the water potentials had fallen to about −1.5 MPa.

Water potentials of the plants were measured with a pressure chamber at the same time of the day within two hours, beginning 7 h after the onset of the light period. When the seedling had been severed at root collar, any perlite that was above the uppermost roots was removed and the pot was sealed with 'Parafilm'. The moisture content of the perlite was determined by weighing the sealed pot immediately after measurements of plant water potential, drying the perlite in 105°C after removing the root system, and reweighing. The fresh weight of the root system was subtracted from the weight of the moist pot contents, assuming that the dry weight of the root system was 20 % of the fresh weight, based on some fresh and dry weight measurements. After discarding nonmycorrhizal, inoculated plants and one with some contamination by *Thelephora terrestris*, 40 mycorrhizal and 49 nonmycorrhizal plants were left.

Regression models of plant water potential were fitted on soil moisture, and the lines for the mycorrhizal and nonmycorrhizal treatments were compared to a single fitted line by means of an F-test (Mead & Curnow 1983). Root tip and mycorrhizal root tip numbers were counted, and shoot and root dry weights and shoot N, P and K concentrations were determined (Chapter 3). The data on plant size and nutrition were bulked for the sequential harvests and subjected to analysis of variance using the days of harvest as blocks.

Experiment 3b had a randomized 2x2 factorial block design with 2 fungal treatments (mycorrhizal and nonmycorrhizal) and 2 watering treatments (well-watered and unwatered). Initially there were 16 replicate plants in each

treatment, and the blocks were different measuring times as the measurements were done on four consequtive days within four hours commencing 6 h after the onset of the light period. Each block consisted of one replicate plant for each treatment, and the order of measuring was randomized within blocks.

Some replicate plants were lost due to lack of mycorrhiza in inoculated plants or contamination of noninoculated plants. The plants to be measured on the first two days were last watered 144 days after germination, and the second half of the plants, 146 days after germination. The watered treatment received an amount of distilled water daily which was estimated to keep the plants well-watered but not to saturate the pots since this would have led to leaching of nutrients. Water vapour and CO_2 exchange were measured with the ADC equipment (Section 3.5.) at 270 µmol $m^{-2}s^{-1}$ radiation when the pots had been unwatered for 10 or 11 days. The seedling in its pot was placed vertically during the measurement, and the needles received radiation from above. Care was taken to measure each plant in the same position relative to the source of light, as the light level was not saturating, and a slight difference in radiation reaching the plant might have led to a difference in the results. After the measurements of gas exchange of a block of 4 plants the water potential and soil moisture content for each plant were measured as in Experiment 3a. Leaf area was measured with a Li-Cor model LI-3100 area meter (Lambda Instruments, Lincoln, Nebraska). The needles were spread on a piece of transparent material, which was passed over the sensor on a moving belt. Number of root tips and mycorrhizal root tips, shoot and root dry weight and shoot N, P, and K concentration of each seedling were determined (Chapter 3).

Some replicate plants were lost due to lack of mycorrhiza in inoculated plants or contamination. In the end, the replicate numbers in each treatment were as follows:

Mycorrhizal watered	Mycorrhizal dry	Non-mycorrhizal watered	Non-mycorrhizal dry
14	12	11	13

Because the replicate numbers were not the same in different blocks, the block design was abandoned in the analysis. In analysis of variance, an angle transformation was used for proportions. If the variances were greater for

large values than small, the analysis was done also with a natural logarithm transformation, as in the cases of stomatal conductance, and soil moisture content. The log transformation did not change the outcome of analysis of variance.

5.3 Results

In Experiment 3a, the dry weights, root tip numbers or root / shoot ratios of the plants were not significantly different in the two treatments, but the shoot N and P concentrations and contents of the mycorrhizal plants were higher (Table 5.1.). This difference was particularly clear in the beginning of the experiment, but it narrowed down later (shown for N % in Fig. 5.1.). The soil moisture decreased with time in the same way in both treatments (Fig. 5.2.).

The shoot water potentials of mycorrhizal plants tended to be more negative than those of nonmycorrhizal ones at a given level of soil moisture (Fig. 5.3.). The two regression models relating soil moisture content and plant water potential were significantly different at 0.05 level. The F-test comparing the regressions is shown below Fig. 5.3.

In Experiment 3b, mycorrhizal infection did not have any significant effect on dry weights or root tip numbers of the plants (Table 5.2.), despite the high percentage mycorrhizas in inoculated plants. The nitrogen and potassium concentrations were very similar too, and mycorrhizal plants had slightly but not significantly more phosphorus in the shoots (Table 5.3.).

Droughting affected the total dry weight accumulation of plants slightly but not significantly (Table 5.2.). Apart from this, the root / shoot ratio was the only growth parameter that showed significant difference: in the dry treatment, this was higher in the nonmycorrhizal plants. The nutrient concentrations were not different in the different treatments, except for the higher phosphorus concentration in the dry treatment. Total shoot contents of N, P, and K were similar in all treatments.

Table 5.1. Characteristics of mycorrhizal *(Paxillus involutus,* ECM) and nonmycorrhizal (NM) Sitka spruce seedlings in Experiment 3a, means ± s.e. * indicates significance of differences between treatments at 0.05 level in analysis of variance, ** at 0.01 level

	Total dwt mg	Root tips	Mycorrhizas %	Root / shoot ratio
ECM	252±13	191± 9	96±1	0.49±0.084
NM	236±13	197±14	0±0	0.47±0.014
Significance of differences of means	ns	ns	**	ns

	N %	P %	K %
ECM	1.86±0.05	0.462±0.027	1.42±0.03
NM	1.68±0.04	0.398±0.016	1.42±0.03
Significance of differences of means	**	*	ns

	N mg	P mg	K mg
ECM	3.20±0.14	0.79±0.058	2.45±0.10
NM	2.66±0.15	0.63±0.040	2.21±0.11
Significance of differences of means	*	*	ns

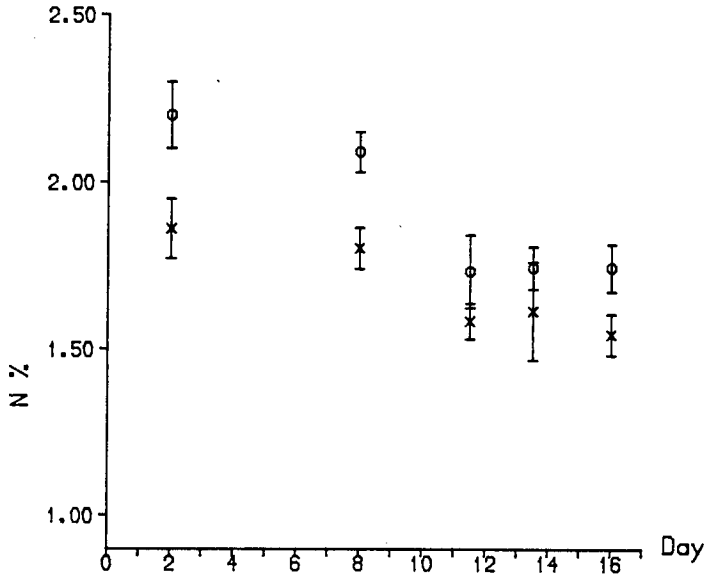


Fig. 5.1. N % in shoots of Sitka spruce seedlings in Exp. 3a. Last watering on day 0. Each point represents 8–12 plants harvested on 2 or 3 days (see text for harvests), means ± s.e. Circle, mycorrhizal with *Paxillus involutus*; cross, nonmycorrhizal.

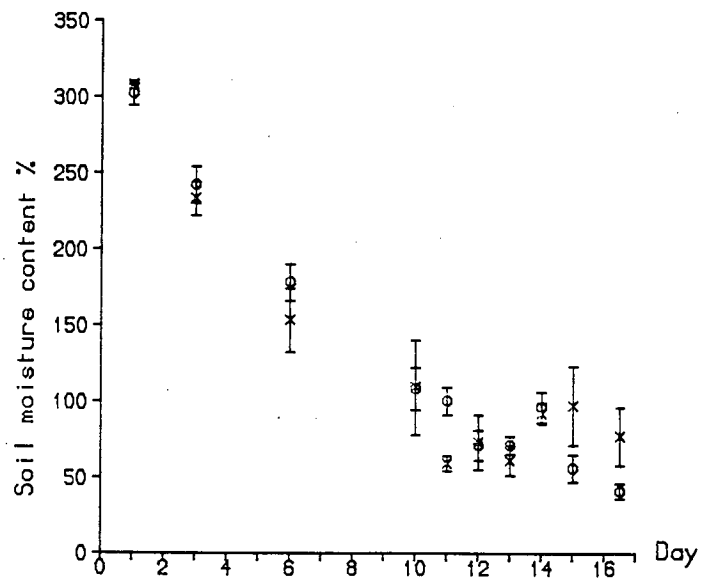


Fig. 5.2. Soil moisture content % in Exp. 3a. Last watering on day 0. Each point is the mean of 4–6 observations, with two standard errors. Circle, mycorrhizal with *Paxillus involutus*; cross, nonmycorrhizal.

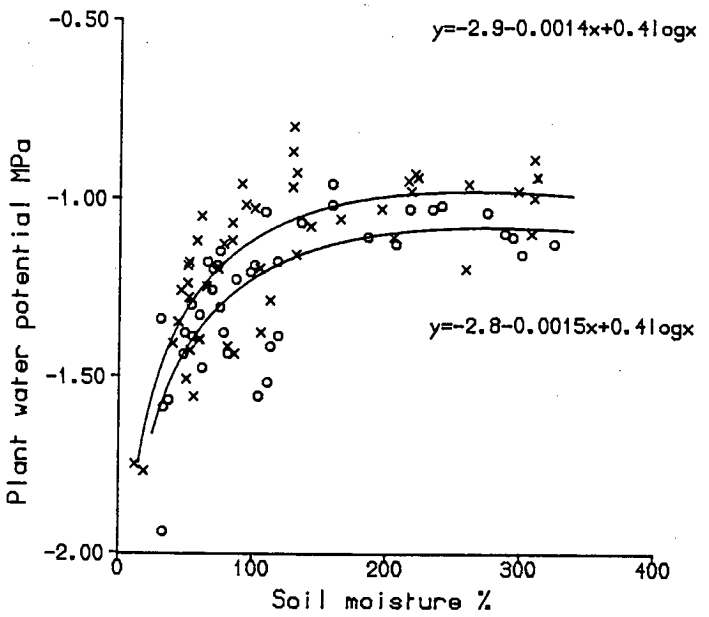


Fig. 5.3. Water potential of mycorrhizal *(Paxillus involutus,* circle) and nonmycorrhizal (cross) Sitka spruce seedlings exposed to drying out in Exp. 3a.

F-test on the significance of the difference between the two regression models. The subscripts 1 and 2 refer to the mycorrhizal (ECM) and nonmycorrhizal treatments, and 'c' refers to the total population, to which the common regression was fitted. RSS = residual sum of squares, MS = mean square, n = number of observations, df = degrees of freedom.

	RSS	n	df	MS
common	1.860	89	86	
ECM	0.761	40	37	
NM	0.866	49	46	
sum	1.627		83	0.0196
difference	0.233		3	0.0780

$$F = \frac{[RSS_c - (RSS_1 + RSS_2)] / df}{(RSS_1 + RSS_2) / df}$$

$F = 0.0780 / 0.0196 = 3.98, \quad p < 0.05.$

Table 5.2. Characteristics of mycorrhizal (*Paxillus involutus,* ECM) and nonmycorrhizal (NM) Sitka spruce seedlings, either well-watered (w) or exposed to drought (d), ± s.e. * indicates significance of differences between main treatments and their interaction (i.a.) at 0.05 level, ** at 0.01 level. If interaction of mycorrhizal and watering treatments is significant, differences between treatment means are indicated with letters (Tukey's test at $p<0.05$).

	Total dwt mg	Leaf area cm^2	Root / shoot ratio
ECM w	400±25	24.2±1.4	0.41±0.012ab
ECM d	354±12	23.2±1.5	0.38±0.019 b
NM w	396±27	24.3±1.4	0.42±0.016ab
NM d	378±32	23.0±1.7	0.46±0.019 a
Significance of differences of means			
ECM-NM	ns	ns	**
w - d	ns	ns	ns
i.a.	ns	ns	*

	Root tips	Mycorrhizas %	Root tips / mg root
ECM w	238±18	85±8	2.08±0.13
ECM d	187±13	96±3	1.98±0.13
NM w	229±28	0±0	1.94±0.18
NM d	228±28	0±0	1.93±0.15
Significance of differences of means and interaction (i.a.)			
ECM-NM	ns	**	ns
w - d	ns	ns	ns
i.a.	ns	ns	ns

Table 5.3. Shoot N, P and K concentrations (% of dry weight) and contents of mycorrhizal (*Paxillus involutus*, ECM) and nonmycorrhizal (NM) Sitka spruce seedlings, either well-watered (w) or exposed to drought (d), ± s.e. * indicates significance of differences between main treatments and their interaction (i.a.) at 0.05 level, ** at 0.01 level.

	N %	P %	K %
ECM w	1.64±0.06	0.229±0.010	1.26±0.03
ECM d	1.73±0.12	0.251±0.006	1.30±0.04
NM w	1.59±0.05	0.217±0.007	1.26±0.03
NM d	1.70±0.09	0.232±0.009	1.29±0.05
Significance of differences of means			
ECM-NM	ns	ns	ns
w - d	ns	*	ns
i.a.	ns	ns	ns

	N mg	P mg	K mg
ECM w	4.65±0.33	0.645±0.041	3.52±0.18
ECM d	4.36±0.19	0.644±0.024	3.32±0.11
NM w	4.37±0.28	0.601±0.041	3.48±0.22
NM d	4.22±0.26	0.580±0.034	3.22±0.19
Significance of differences of means			
ECM-NM	ns	ns	ns
w - d	ns	ns	ns
i.a.	ns	ns	ns

Table 5.4. Shoot water potential (ψ), stomatal conductance, and net photosynthesis rate of mycorrhizal (*Paxillus involutus,* ECM) and nonmycorrhizal (NM) Sitka spruce seedlings, either well-watered (w) or exposed to drought (d), ± s.e., and the corresponding moisture content of perlite. * indicates significance of differences between main treatments and their interaction (i.a.) at 0.05 level, ** at 0.01 level.

	ψ MPa	Soil moisture content %
ECM w	−0.90±0.04	145±10
ECM d	−1.30±0.05	80± 5
NM w	−0.94±0.04	126± 8
NM d	−1.32±0.06	58± 9
Significance of differences of means		
ECM−NM	ns	**
w − d	**	**
i.a.	ns	ns

	Stomatal conductance mmol $m^{-2}s^{-1}$	Net photo-synthesis µmol $m^{-2}s^{-1}$
ECM w	108±17.1	2.47±0.18
ECM d	12± 1.8	0.89±0.11
NM w	135±30.2	2.32±0.22
NM d	15± 3.6	0.95±0.19
Significance of differences of means		
ECM−NM	ns	ns
w − d	**	**
i.a.	ns	ns

The stomatal conductance data showed considerable variation, partly due to the variation in the soil moisture content within treatments. One possible source of error in the determination of stomatal conductance was the fact that the shoot was in a different position during the gas exchange determination than normally, receiving light from a new angle. There were usually no side branches in the seedlings, so most of the shading there was, was caused by the individual needles.

Droughting had a highly significant effect on shoot water potentials, stomatal conductances and net photosynthetic rates (Table 5.4.). The treatment differences for transpiration rates, and the variability of this data, were similar to those for stomatal conductance (data not shown). Mycorrhizal and nonmycorrhizal plants did not show any difference in relation to these variables whether they were droughted or not. However, the average soil moisture content was significantly lower in the nonmycorrhizal treatment than in the mycorrhizal one (Table 5.4.). The soil moisture content was relatively low in the watered treatment.

The relationship between stomatal conductance and net photosynthetic rate was not different in mycorrhizal and nonmycorrhizal plants (Fig. 5.4.).

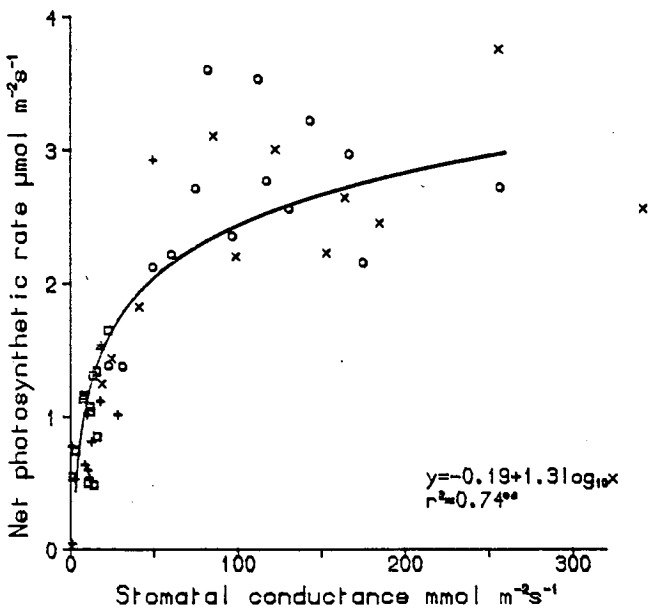


Fig. 5.4. Relationship of stomatal conductance and net photosynthetic rate of mycorrhizal and nonmycorrhizal Sitka spruce seedlings in Exp. 3b. Circle, mycorrhizal watered; square, mycorrhizal droughted; cross, nonmycorrhizal watered; plus, nonmycorrhizal droughted.

5.4 Discussion

5.4.1 Mycorrhizas, nutrition and growth of plants

In these experiments, there was little mycorrhizal effect on plant growth despite the very high mycorrhizal percentage and despite the fact that the N and P concentrations of mycorrhizal plants were higher in the beginning of the first experiment (Exp. 3a); nutrition apparently was not a growth-limiting factor in these conditions. As nutrients were depleted from the pots during the experiments, the difference between mycorrhizal and nonmycorrhizal plants in N and P concentrations narrowed down. This is consistent with results from several other short-term experiments showing that mycorrhizal structure is not always beneficial in terms of growth and nutrient uptake if the plants are provided with high enough amounts of soluble nutrients (Bowen 1973) and if rooting density is not a factor limiting nutrient uptake (Alexander & Fairley 1986). In some cases, mycorrhizal infection has decreased the growth of coniferous seedlings growing in a confined space (Section 2.3.1.); the mycorrhizal fungus is a sink of carbon to the host plant, and if there is no advantage in terms of nutrient uptake, the mycorrhizal structure may lead to altered distribution patterns of carbon without an increase in overall growth (Section 2.3.1.). Nevertheless, there always remains an inherent difference in the morphology of mycorrhizal and nonmycorrhizal root systems in the amount and quality of absorbing surface.

The drought treatment did not significantly affect the growth of plants either, even though the dry weights of droughted plants in Experiment 3b were slightly lower than those of the watered plants. The higher mean root / shoot ratio of nonmycorrhizal plants compared to mycorrhizal in the dry treatment was probably coincidental rather than a reaction to the drought treatment, as there was no difference in the root / shoot ratios of different plants in Exp. 3a.

The only significant difference in the nutrition of the watered and droughted plants in Exp. 3b was the higher phosphorus concentration of the water stressed plants. This is likely to have been the result of a dilution effect in the slightly larger watered plants, as the total contents were not significantly affected by the watering treatment. It is of note that this effect was opposite

to that in Experiment 1, in which the unwatered plants tended to have lower nutrient concentrations, particularly N and K, than watered controls. This difference between the two experiments may have been due to the different substrate used. In Experiment 1 this was vermiculite–peat, which is closer to real soil than is perlite, in that it has a substantial cation exchange capacity, and it also contains some nutrients in organic form in the peat component. On the contrary, all nutrients in the pots in the present experiments were in a soluble form in the 'soil' solution. Therefore the drying treatment was less likely to reduce nutrient availability enough to affect nutrient uptake to a large extent, especially as the rooting density was quite high. Hence a reduction in nutrient uptake was probably not a reason for the slightly reduced growth of the seedlings during this experiment.

5.4.2 Physiological effects of drought, mycorrhizas and nutrition

Comparison of Experiments 1 and 3 shows the necessity of controlling the nutrition of plants, if mycorrhizal effects other than nutritional ones are of interest. Some of the results were actually reversed when comparable size and nutrition of all experimental plants was obtained, as opposed to conditions with a strong mycorrhizal effect on nutrient uptake and growth. Now the shoot water potentials of mycorrhizal seedlings were lower than nonmycorrhizal, whilst they were higher in Experiment 1a (Chapter 4), and there was little difference in the stomatal conductances and photosynthetic rates of the watered controls, which were higher in mycorrhizal seedlings in Experiment 1a. Moreover, within the limitations of the data in each experiment, the relationship between stomatal conductance and net assimilation rate in mycorrhizal and nonmycorrhizal plants was different in Experiment 1, suggesting a nutrient effect on the photosynthetic apparatus, whereas in Experiment 3 there was no difference between the treatments in this respect. The overall stomatal conductances and net photosynthetic rates were lower than those of the mycorrhizal plants in the beginning of Experiment 1, partly because of the lower light level at which the measurements were done. Also, the drought treatment was more severe, and the stomatal conductances in the dry treatment were very low even though net photosynthesis was still positive.

Other research does not give much evidence of ectomycorrhizal effects on photosynthesis either, except for nutritional effects, as reviewed in Section 2.3.

Parke *et al.* (1983) did find considerably higher net assimilation in water stressed mycorrhizal Douglas fir *(Pseudotsuga menziesii)* as opposed to nonmycorrhizal, but not before the plants had been exposed to a number of drying and rewatering cycles. When mycorrhizal and nonmycorrhizal plants were compared after a suddenly imposed drought treatment, mycorrhizal infection as such did not confer any advantage to the host in terms of gas exchange.

The movement of water in the soil-plant-atmosphere continuum may be treated using an Ohm's law analogue (Kramer 1983). Despite its limitations, this can be a useful model to facilitate the understanding of the process. In a simple form:

E = water flow rate,

$\Delta\psi$ = difference between water potentials along the pathway of water,

L_p = conductance to water in the soil-plant system

$$L_p = \frac{E}{\Delta\psi}$$

$\Delta\psi$ is taken as $\psi_{shoot} - \psi_{soil}$

In Exp. 3b, both in the watered and droughted treatments, the soil was drier with nonmycorrhizal plants but the water potentials were not different from those of mycorrhizal plants, hence the $\Delta\psi$ was larger in the mycorrhizal treatment. This is confirmed by the result of Exp. 3a showing that the water potentials of mycorrhizal plants were lower at the same soil moisture content. As the flow rates were not different, the combined soil+plant conductance in the mycorrhizal systems was somewhat lower. In contrast, in Experiment 1 the water potentials of mycorrhizal plants were higher if the soil moisture was the same as for nonmycorrhizal plants, and consequently the conductance higher for mycorrhizal systems, as stomatal conductances tended to be higher in mycorrhizal plants. This difference between the experiments suggests that the mycorrhizal effects in Experiment 1 were mostly due to extension of roots and mycelium in the soil, and the better P and K status of mycorrhizal plants, as already discussed in Sections 4.4.1. and 4.4.2. However, the hypothesis that ectomycorrhizal structure as such increases water uptake has not obtained experimental support from this work, but rather the opposite.

Other research has shown lower water potential in water stressed ectomycorrhizal Douglas fir (Parke *et al.* 1983), and lower water potential with consequently decreased soil+plant conductance to water flow in ectomycorrhizal radiata pine plants than nonmycorrhizal (Sands & Theodorou 1978). In the experiments in which ectomycorrhizal infection has increased transpiration or water potential, there is usually a possibility that this was caused by either nutritional effects or hyphal extension (Dixon *et al.* 1980, 1983, Boyd 1987, Boyd *et al.* 1986, Osonubi 1989). Hence it seems possible that ectomycorrhizal structure can decrease the conductance of the pathway of water from soil to the plant, if these effects are excluded. Determining the location of the lowest conductance requires further work. The possibilities include lower conductance between the bulk soil and the soil–root interface; lower conductance at the soil–root interface (higher contact resistance); and lower root conductance.

Before discussing the most likely location of low conductance, the possibility of mycorrhizal root systems having smaller amounts of effective absorbing surface in experiments with mycorrhizal plants showing reduced water uptake needs to be examined. Generally, the major differences between the architecture of ectomycorrhizal and nonmycorrhizal pine and spruce rootlets are the check of longitudinal growth and increased diameter of mycorrhizal roots. Hence it is possible that nonmycorrhizal plants can have a greater length of root per unit volume in a confined rooting space, and therefore the apparently increased conductance would be due to larger effective absorbing surface in nonmycorrhizal root systems. This is counteracted by the relative thickness of mycorrhizal roots, which increases the absorbing surface, as the extraradical mycelium does, too. In one of the present experiments (Exp. 3b) the nonmycorrhizal plants had larger root / shoot ratios than mycorrhizal plants, which may have been the reason for the lower water content in the substrate. However, the low water potentials of mycorrhizal plants in Exp. 3a were not associated with differences in root / shoot ratios or soil moisture compared to the mycorrhizal treatment. The absorbing surface of the mycorrhizal plants was increased by their abundant external mycelium instead. On the contrary, the mycorrhizal radiata pine seedlings of Sands & Theodorou (1978) did not have well–developed strand systems, and their root / shoot ratio was slightly larger than that of nonmycorrhizal plants (0.79 for mycorrhizal, 0.72 for nonmycorrhizal, calculated from means presented). It is interesting

therefore that the result obtained was the same in both experiments in spite of the differences in the root systems: the main difference between the inoculation treatments was the lower shoot water potential of mycorrhizal seedlings. Therefore, even if there was a difference in the absorbing surface area, it is likely that some other factor caused differences as well. Unfortunately the effective absorbing surface area of root and mycelial systems is nearly impossible to accurately measure for comparisons of mycorrhizal and nonmycorrhizal plants, although estimates of this have been made (Boyd 1987).

Regarding their results on radiata pine referred to above, Sands & Theodorou (1978) concluded that the lower soil-to-plant conductance in mycorrhizal plants was largely caused by a difference in soil resistance, which could be due to a difference in root geometry. The soil component of the combined soil+plant resistance to water, as predicted from models of water flow in the soil-plant-atmosphere continuum, is usually very small, but it can be considerable with sparsely rooted plants growing in coarse soil such as the pine plants in the experiment by Sands & Theodorou (1978). In the present study, the substrate was coarse, which might lead to low soil conductance, but as the rooting density was as high as 3 root tips cm^{-3} (as opposed to ca. 0.3 in the pots of the radiata pine plants), this would make water potential gradients in the soil less important (Williams 1974). The importance of the soil resistance has been questioned, as large potential gradients around roots seem to be restricted to conditions of unrealistically low rooting densities and high extraction rates from dry soil (Williams 1974 Passioura 1988), and low water uptake rates by seedlings in coarse soil have instead been explained by high contact resistance, which could involve root shrinkage or soil shrinkage as well as drainage of large pores (Passioura 1988, Örlander & Due 1986). Mycorrhizas with mycelium should be expected to be beneficial in maintaining contact to soil (Dosskey & Ballard 1980); even though there is little direct evidence of better functional contact between soil and mycorrhizal mycelium as opposed to roots, the growth of mycelium around soil particles can readily be seen under a dissecting microscope. These facts do not favour the argument that the major decrease in conductance would be in the soil or at the soil-root interface in the present experiment.

Within the plant, the largest drop of water potential is usually from the root surface to the xylem, whilst the pathway between root xylem and the shoot is of high conductance (Passioura 1982). Therefore a possible location of a low

conductance in mycorrhizal plants is in the sheath and root cortex. Direct measurements of hydraulic conductances of roots of *Pinus taeda* (Sands *et al.* 1982) and root systems of *Pseudotsuga menziesii* (Coleman *et al.* 1987) with varying degrees of mycorrhizal infection have either shown no mycorrhizal effect (Sands *et al.* 1982) or a lower conductance in heavily mycorrhizal root systems as opposed to ones with fewer mycorrhizas (Coleman *et al.* 1987). Because phosphorus fertilization increased the root conductance, Coleman *et al.* concluded that the mycorrhizal effect was independent of, and opposite to nutritional effects. The ways in which mycorrhizal structure could affect root conductance and the implications of this are discussed further in Chapter 9.

More efficient osmotic adjustment was suggested by Parke *et al.* (1983) for their mycorrhizal Douglas fir seedlings subjected to cyclic drying and rewetting which had lower water potentials than nonmycorrhizal seedlings; as the photosynthetic rates of the nonmycorrhizal plants were very low, this could have led to depletion of their carbohydrate reserves, and therefore lack of osmotically active compounds. The low water potentials of mycorrhizal plants in the present experiments cannot be explained in this way as the mycorrhizal effect was obvious in well-watered plants as well as drought treated ones (Exp. 3a). Moreover, the net photosynthetic rate was similar in both inoculation treatments. For the hypothesis of Parke *et al.* (1983) to gain support from these experiments, the water potentials of the mycorrhizal plants should have been much lower in Experiment 1, in which the carbohydrate reserves of nonmycorrhizal plants could be postulated to have been low (Chapter 4).

Nevertheless, the possibility of different tissue water relations in mycorrhizal and nonmycorrhizal plants in Exp. 3 cannot be excluded, especially as the growth of plants was similar in both treatments. If the water potential of mycorrhizal plants had been lower because of lower turgor pressure in the needles, their growth might have been affected. In particular, leaf area was similar in both treatments, even though extension growth is the first growth component to be affected by lower turgor (Chapter 2). The differences between mycorrhizal and nonmycorrhizal plants were small, however, and perhaps therefore did not lead to differences in the growth of the plants. Small differences like this are most likely to be overridden by other mycorrhizal effects in nursery and field conditions, especially when longer time periods are considered.

CHAPTER 6
EFFECTS OF PLANT NUTRITION ON TISSUE WATER RELATIONS

6.1 Introduction

The results of Experiment 1 indicated a generally lower water potential in nonmycorrhizal than mycorrhizal plants, nonmycorrhizal plants having very low phosphorus and potassium levels. However, when the same measurements were taken on plants with no difference in their nutrient status, the effect was reversed: the water potentials of mycorrhizal plants were slightly more negative than those of nonmycorrhizal plants. Hence it is possible that improved nutrition is the most powerful way in which mycorrhizas affect plant water relations. Because there is little information on the effects of nutrition on water relations of coniferous seedlings (Section 2.2.1.), it would seem logical to develop an understanding of these effects whilst attempting to elucidate the mycorrhizal role in water relations.

Total water potential is not always a good indicator of differences in the water relations of plants, such as the mycorrhizal and nonmycorrhizal seedlings in Experiments 1 and 3. The osmotic pressure may vary, and therefore the turgor pressure of tissues at the same water potential may be different. The relations of turgor and osmotic pressures as well as tissue elasticity have been reported to vary with a number of factors, including ontogeny of the tissue (Tyree *et al.* 1978), season (Tyree *et al.* 1978, Parker *et al.* 1982, Ritchie & Shula 1984, Colombo 1987, Grossnickle 1988b), water stress history (reviewed by Morgan 1984 and Abrams 1988) and salinity of the soil (Radhi 1985). Studies of nutritional effects on tissue water relations are rare, apart from those by Radin and coworkers (Section 2.2.1.). This lack of information is particularly surprising in view of the many possible ways in which nutrition could affect cell and tissue water relations, as pointed out by Tyree & Jarvis (1982). Adequate nutrition may be expected to be a prerequisite for osmotic adjustment, and it may affect tissue elasticity. Many of the osmotically active 'compatible' compounds (non-toxic, water-soluble osmotica carrying no net charge, which are not harmful for metabolism in large concentrations) such as amino acids are nitrogenous, and energy is required for the synthesis of large quantitities of

osmotically active substances and holding them within the cells (Tyree & Jarvis 1982). It has also been suggested that the decreased root conductance in N and P deficient plants may be a primary reason for reduced expansion growth of leaf cells, as these plants cannot keep up adequate turgor for growth (Radin & Boyer 1982, Radin & Eidenbock 1984). In the case of K deficiency, there may additionally be a more direct effect, as K^+ is one of the major osmotic constituents in cells. K^+ and Cl^- were found to be among the main components in osmotic adjustment in mung bean *(Vigna mungo* (L.) Hepper) seedling roots alongside amino acids and sugars, even though they were less important in the hypocotyl (Itoh *et al.* 1986, 1987).

To examine the effects of nutrition on tissue water relations, Experiment 4 was conducted on watered mycorrhizal Sitka spruce seedlings grown at two levels of balanced nutrition.

6.2 Materials and methods

Sitka spruce seedlings were grown in 'Ray Leach' tubes in perlite as described for Experiment 2. In this experiment (Exp. 4), the plants were inoculated with *Paxillus involutus* and grown further. Up to the age of 4 months they were watered with an excess of 20 mg N l^{-1} Ingestad solution five days a week, then for one month with 30 mg N l^{-1} solution. At this stage the plants were randomly allocated in the high-nutrient and low-nutrient treatments. The high-nutrient treatment was fed with solution containing 70 mg N l^{-1} and the low nutrient treatment with 40 mg N l^{-1} five days a week for three months, after which the low-nutrient treatment received only tap water for two weeks. On the days when the plants were not fed, they were watered with tap water.

The experiment commenced when the plants were 9 months old, and the measurements of 7 replicate plants per treatment were completed within 15 days. The day before excision each plant was watered with distilled water in an attempt to reduce direct osmotic effects on tissue water relations.

Pressure-volume analysis was performed on 8-9 cm long apical parts of the shoots. The procedure was applied from Ritchie (1984) as follows, and the analysis of pressure-volume curves followed Hellkvist *et al.* (1974) and Tyree & Jarvis (1982).

The night before the measurements the part of the stem which would protrude from the pressure chamber was defoliated, the shoot was wrapped in several layers of 'Clingfilm', and cut off with a razor blade. The shoot was recut under water, and left to rehydrate in distilled water in a cool place (10–15°C) covered with aluminium foil to prevent transpiration.

After ca. 12 hours the specimen was blotted dry and weighed. This weight was not always an accurate measure of the turgid weight because of the difficulty of completely drying the surface, and therefore it was not used in the calculations, but only as a guideline for discarding samples if it deviated too much from the sum of expressed sap and fresh weight of the specimen immediately after the procedure (see below). The seedling was rewrapped in 'Clingfilm', and placed in a pressure chamber lined with damp tissue paper. The first balance pressure was recorded, between 0.04...0.10 MPa. If this was more than 0.10 MPa, the seedling was discarded, as it was not considered adequately hydrated. The pressure was raised by 0.5 MPa above the balance, and sap was collected for 10 minutes in pre-weighed pieces of tubing filled with tissue paper. Before the next reading the specimen was allowed to equilibrate for 15 minutes at the previous balance pressure. In all, 10–11 balance readings were taken. The seedling was removed from the chamber, weighed, and the dry weights of the needles and the stem were measured after drying for 72 h in 85°C. Needle N, P, and K concentrations of three ca. 100 mg subsamples for each plant were determined.

In the calculations, the density of the sap was assumed to be 1.000 g cm^{-3}. The following symbols were used.

Measured variables:

dwt = dry weight of the specimen mg

twt = turgid weight (fresh weight after the procedure + total expressed sap) mg

v_e = cumulative weight of expressed sap mg

Calculated variables:

R^* = relative water content

ψ_p = turgor pressure MPa

ψ_s = osmotic pressure MPa

B = bound water content, or apoplastic volume (part of R^*)

F = free water content, or relative symplastic water content

ε = bulk modulus of elasticity MPa

ε_{max} = maximum bulk modulus of elasticity

The relative water content R^* was calculated as

$$R^* = 100 \times \frac{(twt - v_e) - dwt}{twt - dwt}$$

If the measured cumulative water loss from the plant during the procedure was less than 90 % of the difference in plant weights, the sample was discarded.

Pressure-volume curves were drawn by plotting the reciprocal of the pressure against R^*. Linear regression was fitted to the end part of the curve, with r^2 of 0.998 or more. The intercept of the line with the y-axis yielded an estimate of osmotic pressure at full turgor, and the relative volume of apoplastic water (B) was estimated as the intercept with the x-axis. Turgor loss point was estimated from the graphs as the point where the curve turned linear. A typical pressure-volume curve is shown in Fig. 6.1.

The relative free water content was calculated as:

$$F = 100 \times \frac{R^* - B}{100 - B}$$

The bulk modulus of elasticity, ε, was calculated as

$$\varepsilon = \frac{\psi_{p1} - \psi_{p2}}{F_1 - F_2}$$

The maximum bulk modulus of elasticity was taken as ε between the two highest free water contents measured, as ε was always largest at high free water contents (Fig. 6.2.).

Differences between the means were tested with a t-test.

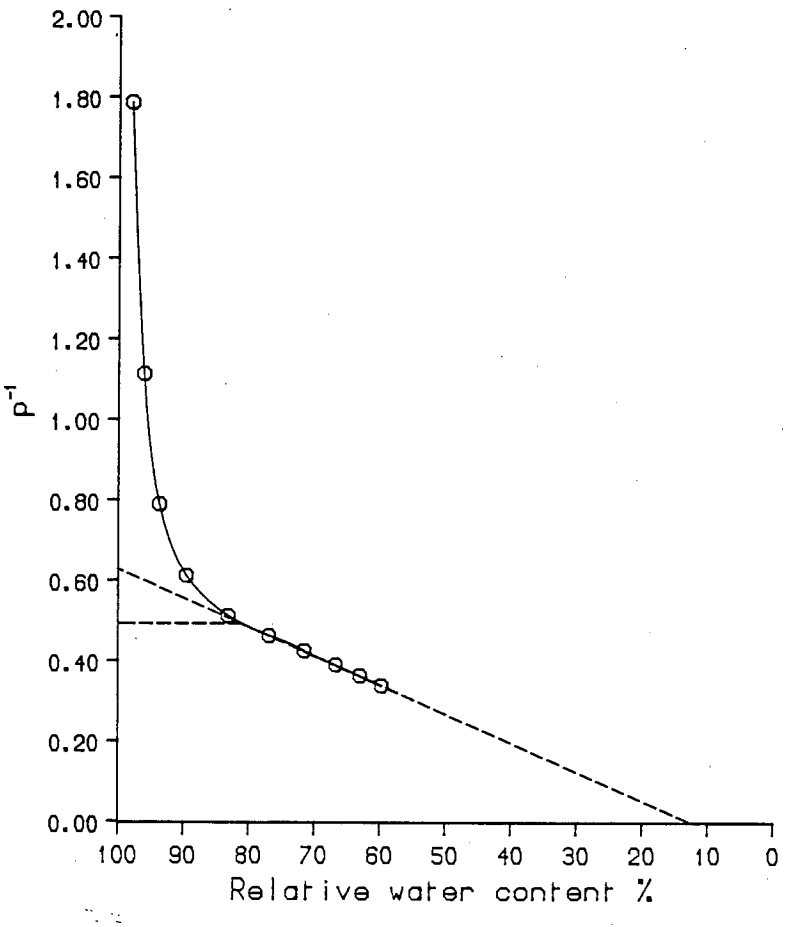


Fig. 6.1. A typical pressure-volume curve from Exp. 4. Extrapolation of the linear part to x-axis yields an estimate of the relative apoplast volume (B), and to y-axis, reciprocal osmotic pressure at full turgor. Turgor loss point determined as the point where the curve turns linear.

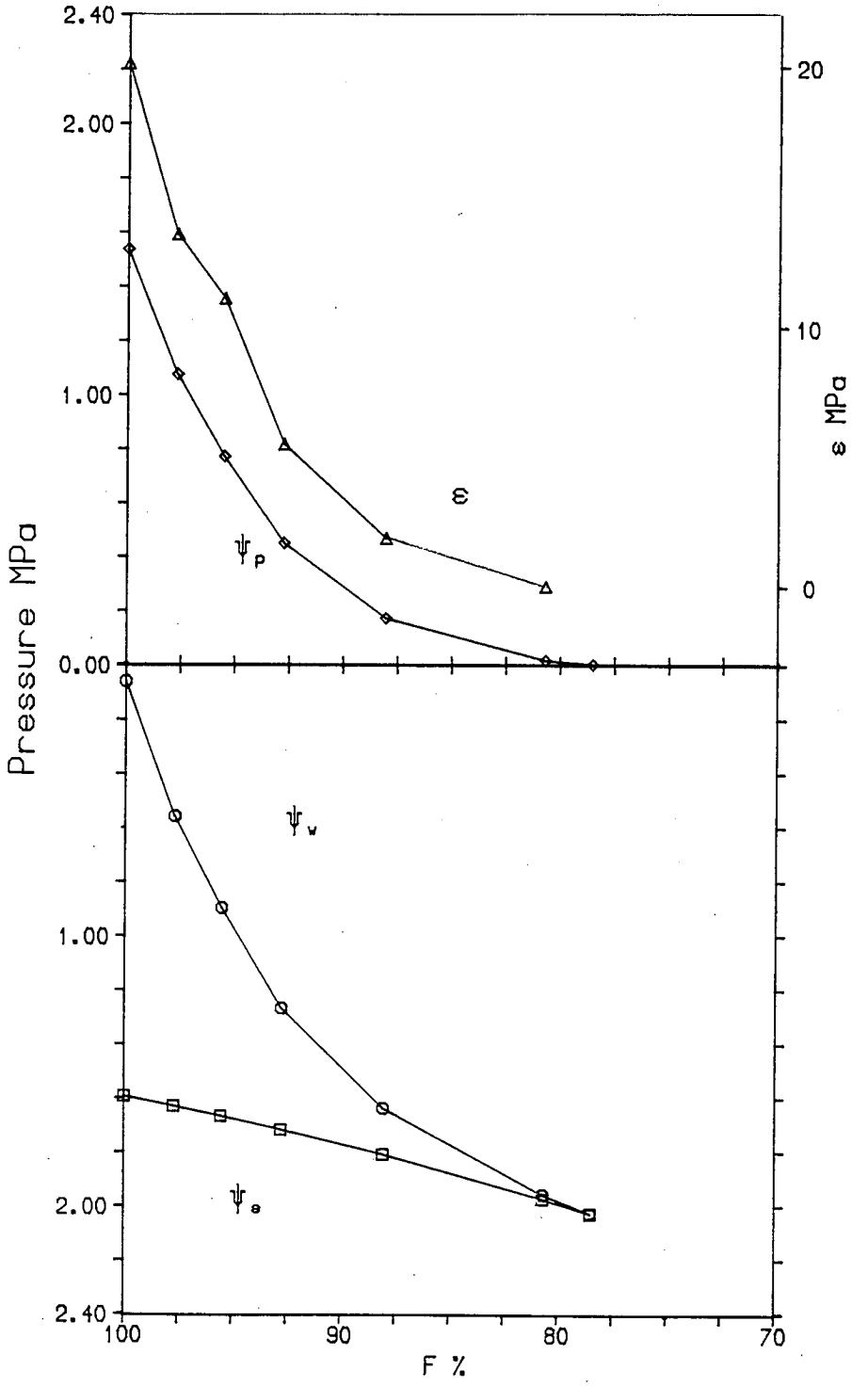


Fig. 6.2. A typical Hoeffler diagram from Exp. 4 showing the changes in water potential (ψ_w), osmotic pressure (ψ_s), turgor pressure (ψ_p) and bulk modulus of elasticity (ε) with relative symplastic water content (F).

6.3 Results

The N, P, and K concentrations in the two treatments were significantly different at 1 % significance level (Table 6.1.), but the dry weight of the shoot was only slightly higher in the high nutrient regime. The proportion of the stem in the specimens was the same in both treatments, and therefore this did not cause uncertainty in the results (Parker & Pallardy 1988).

Table 6.1. Needle N, P and K concentrations (% of dry weight) and the proportion of stem in the p/v-analysis sample, and dry weight of the whole shoot of 9-month-old Sitka spruce seedlings grown in a high (7 replicate plants) and low (5 replicate plants) nutrient regime, means ± s.e. ** indicates significance of differences between treatments at 0.01 level (t-test).

	N %	P %	K %
High nutrient	1.80±0.09	0.286±0.016	1.19±0.05
Low nutrient	1.09±0.04	0.201±0.012	0.88±0.03
Significance of differences of means	**	**	**

	Stem %	Shoot dry weight g
High nutrient	13.8±0.9	1.59±0.099
Low nutrient	13.8±0.7	1.50±0.069
Significance of differences of means	ns	ns

Table 6.2. Tissue water relations of 9-month-old Sitka spruce seedlings grown in a high (7 replicate plants) and low (5 replicate plants) nutrient regime, means ± s.e. * indicates significance of differences between treatments at 0.05 level (t-test).

	ψ_s turgid, MPa	ψ_s at turgor loss point, MPa
High nutrient	-1.67±0.026	-2.20±0.041
Low nutrient	-1.73±0.019	-2.23±0.039
Significance of differences of means	*	ns

	R^* at turgor loss point %	Apoplastic water content %
High nutrient	79.0±0.7	12.4±1.4
Low nutrient	79.1±0.3	12.3±1.0
Significance of differences of means	ns	ns

	ε_{max} MPa	Dry / turgid weight ratio %
High nutrient	19.6±0.66	27.4±2.6
Low nutrient	18.3±0.89	30.2±1.8
Significance of differences of means	ns	ns

The osmotic pressure at full turgor was significantly lower in the low nutrient than in the high nutrient treatment, even though the difference of the means was no more than 0.06 MPa (Table 6.2.). The means of osmotic pressure at turgor loss point were not significantly different, even though this was lower for the low nutrient treatment corresponding to the osmotic pressure at full turgor. There is inherent uncertainty in determining the turgor loss point as the point where the pressure-volume curve turns linear, resulting in greater variation for this parameter than the osmotic pressure at full turgor. The means of relative water content at turgor loss point, relative apoplastic water content, maximum bulk modulus of elasticity and the ratio of dry weight to turgid weight did not differ between the treatments.

6.4 Discussion

The osmotic pressures at full turgor, about -1.7 MPa, and at turgor loss point, about -2.2 MPa, were in the range found for several species of coniferous seedlings (Kandiko *et al.* 1980, Rees & Grace 1981, Ritchie & Shula 1984, Colombo 1987). These parameters have shown marked seasonal variation, being highest in the spring during rapid growth in Douglas fir (Ritchie & Shula 1984) and Norway spruce (Tyree *et al.* 1978). The seedlings in this experiment had been grown in glasshouse for an extended growing season, and their osmotic pressures were similar to those measured for black spruce *(Picea mariana* (Mill.) B.S.P.) when their second year shoot elongation was complete (Colombo 1987), even though the maximum bulk modulus of elasticity was higher in the black spruce than in the present experiment. However, the ε_{max} here was similar to values found by Ritchie & Shula (1984) in the autumn, when this was at its highest. Similarly, Tyree *et al.* (1978) found larger ε in mature leaves of several tree species compared to those still expanding. A high bulk modulus of elasticity reflects a large change in turgor for a given change in water content, hence low tissue elasticity (Tyree & Hammel 1972).

The treatment differences - or the lack of them - in the present study must be seen against the background of seasonal changes in tissue water relations, for seedlings sampled during a period of rapid shoot elongation might have shown a stronger nutrient effect. However, in experiments with nutrition, there is a risk of the results becoming confounded by differences in plant size and

growth rates which is greatest at the time of exponential growth.

The N, P and K concentrations of the high nutrient treatment were within, or slightly higher than the range found in healthy 1+1 Sitka spruce transplants in nurseries (Benzian & Smith 1973). The low nutrient treatment showed N concentrations below deficiency limits as defined by the Forestry Commission for Sitka spruce stands over 0.3 m high, but P and K were in the optimum range (Binns *et al.* 1980). However, as these seedlings were younger, somewhat higher concentrations would be expected. At these nutrient concentrations, and with little difference in shoot size, the only significant difference in the water relations parameters was the lower osmotic pressure in the low-nutrient treatment. It is of note that this cannot have been a straightforward adjustment to substrate osmotic pressure, as in the case of adjustment to soil salinity in salt tolerant species (Radhi 1985), because the osmotic pressure was lower in the plants fed with the more dilute nutrient solution. Wilson & Ludlow (1983) hypothesized a direct effect of high soil and foliar K concentration on cell water relations, and attempted to induce osmotic adjustment in tropical grasses by fertilizing them to high K concentrations. This did lead to lower osmotic pressures in watered plants, but was not associated with more extensive osmotic adjustment during drought, but promoted leaf death. The mechanisms involved in nutritional effects can be expected to be rather more complex than in adjustment to salinity.

Lower osmotic pressures have also been found in N deficient wheat plants *(Triticum aestivum;* Morgan 1986) and cotton plants *(Gossypium hirsutum;* Radin & Parker 1979a), but this did not lead to more efficient osmotic adjustment during dry conditions (Radin & Parker 1979b). Therefore the lowered osmotic pressure as such cannot be considered an advantage for the plants (Tyree & Jarvis 1982). The lower tissue elasticity found in N deficient wheat and cotton plants was not observed here. If any differences in ε were concomitant with lower osmotic pressures, they were small relative to variation.

As the main difference in the nutrition of mycorrhizal and nonmycorrhizal plants in Experiment 1 was the low P status of nonmycorrhizal plants, and in the present experiment both nutrient treatments had same proportions of all nutrients in the solution, direct inferences about the low water potentials of nonmycorrhizal seedlings in Experiment 1 cannot be made. However, the

results do tentatively lend support to the suggestion made in Chapter 4 that the mycorrhizal effect found there was primarily a nutrient effect, as a slightly lower nutrient regime here resulted in slightly lowered osmotic pressure. Also, lower water potentials, possibly due to low root conductance, have been found in other P deficient plants (Radin & Eidenbock 1984). Correspondingly, the lower water potentials of mycorrhizal plants compared to nonmycorrhizal plants in Experiment 3a would not have been due to the small difference in plant nutrition, as the N and P concentrations were higher rather than lower in the mycorrhizal plants. Plants for Exp. 3 were grown in similar substrate as the plants in Exp. 4 reported in this chapter, and therefore the results are readily comparable.

CHAPTER 7
EFFECTS OF REPEATED DRYING CYCLES ON NUTRITION AND ALLOCATION OF GROWTH IN DIFFERENT MYCORRHIZAL PLANTS

7.1 Introduction

As no major differences in the drought resistance of mycorrhizal and nonmycorrhizal Sitka spruce plants could be found in conditions with a limited rooting volume, the possibility still remained that mycorrhizas could increase the drought resistance of plants by extending their root systems to a larger substrate volume than nonmycorrhizal either by means of stimulating root growth or growing extraradical mycelium. Furthermore, sustained root growth and nutrient uptake by mycorrhizal root systems during prolonged or repeated drought could be an explanation of the fact that mycorrhizas can be beneficial in the long term even if they have no effect in the short term (Parke *et al.* 1983).

Droughting generally reduces root growth, but not in proportion to the reduction in shoot growth, which usually results in an increase in the root / shoot dry weight ratio (Kramer 1983). This was observed in Experiment 1, in which droughting had an after-effect on the allocation of growth, as the roots grew relatively more than shoots after rewatering. Another finding was that the initiation of root tips was more severely affected by the drought treatment than was root dry weight or length of longest roots, even though the root tip numbers recovered rapidly after rewatering. Because of the differences in the size and nutrition of mycorrhizal and nonmycorrhizal plants in Exp. 1, the results could not be fully exploited to assess the role of mycorrhizal infection in terms of root growth during drought. Therefore Experiment 5 was designed to study whether mycorrhizal and nonmycorrhizal plants would react in a different way to repeated drying and rewatering cycles in terms of allocation of growth between roots and shoots, formation of root tips, depth distribution of root, and mineral nutrition.

7.2 Materials and methods

A randomized 3 x 2 factorial block design was used, with three fungal treatments and two water regimes, and with 22 blocks with one replicate plant for each treatment per block. Sitka spruce seedlings were sown in perlite in 65 cm^3 tubes and kept in a glasshouse with a minimum temperature of 20°C, covered with a polythene propagator lid in an attempt to prevent contamination with airborne spores. Because the plants were grown in the summer, no additional lighting was necessary. The seedlings were watered daily with Ingestad solution of 15 mg N l^{-1}, and inoculated with liquid cultures of *Paxillus involutus* or *Thelephora terrestris* or left uninoculated (Section 3.1.). Twenty-six days after inoculation (50 days after germination) the seedlings were transplanted to 170 cm^3 'Ray Leach' tubes (Plate 4.1.) lined with a bag of polythene so that the substrate and the root system could be pulled out intact. If necessary, plants were reinoculated at transplanting. Plants were transferred to a growth room with 18°C day and night, 16 h photoperiod, 70 % relative humidity (0.62 kPa vapour pressure deficit) and PAR 250-350 µmol $m^{-2}s^{-1}$. At this stage the experiment was randomized and the blocks were arranged as rows across of the growth room bench, because the variation in light intensity was small across the bench (within blocks) compared to that with the length of the bench (between blocks). The amount of perlite at the top of each pot was checked and adjusted to an approximately 1 cm thick layer covering the uppermost lateral roots. After a 45 day period of recovery and acclimation, half of the plants were exposed to three 5 day drying cycles with 5 day recovery time in between. Hence the treatments began 95 days after germination. The control plants were watered with distilled water during the drying cycles, and all plants were watered daily with Ingestad solution of 70 mg N l^{-1} for five days before the first drying cycle, and during the 5-day recovery periods.

The height of each seedling was measured from the base of the lowest needles to the tip of the upper needles with a ruler, five times with varying intervals during the drying and rewetting cycles, and at the final harvest. Relative height growth rates (RGR) were calculated as in Section 4.2.

To provide an indication of the severity of the drought treatment, whole-plant transpiration rates of half of the plants (in alternate blocks) with *Thelephora terrestris* were measured gravimetrically (Section 3.3.) during the final drying

cycle and for the three days from rewatering, every other day, beginning 9 h after the commencement of the light period.

A destructive harvest took place 3 days after the last rewatering. The shoots were severed at the root collar, and the root systems were deep frozen (-18°C) with their pots intact. The frozen perlite with the root system was removed from the pot and cut in three with a handsaw: top to 5 cm, 5-10 cm, and 10-15 cm. Root tips and mycorrhizal tips were counted and root dry weights determined separately from each part. Shoot dry weights and N, P and K concentrations were determined as described in Chapter 3.

Because of contamination by airborne *Thelephora terrestris* and scarce colonization by *Paxillus involutus*, most blocks did not contain all treatments. Therefore the block design was abandoned in the analysis, and treatment means were compared to each other with t-tests.

7.3 Results

Relative shoot height growth rates of the plants (Fig. 7.1.) were always lower in the droughted treatments than in their respective controls. *Paxillus involutus* mycorrhizal plants had a lower RGR than *Thelephora terrestris* mycorrhizal or nonmycorrhizal plants in both droughted and well-watered treatments, although the RGR of *Thelephora* plants declined towards the end of the experiment relative to other treatments.

At the end of the experiment, mycorrhizal plants were slightly larger than nonmycorrhizal. All dry weights were reduced by drought, those of *Paxillus* mycorrhizal plants more than others (Fig. 7.2.).

The root / shoot dry weight ratio was increased from 0.63 to 0.73 in the nonmycorrhizal plants whereas *Thelephora* mycorrhizal plants were unaffected by droughting (Table 7.1.). The extent of mycorrhizal infection was around 70 %, and was not affected by drought or fungal species (Table 7.1.).

Because of the large variation in the amounts of root found in the lowest part of the pots (10-15 cm), data from the two lowest sections of the root systems were combined in the analysis.

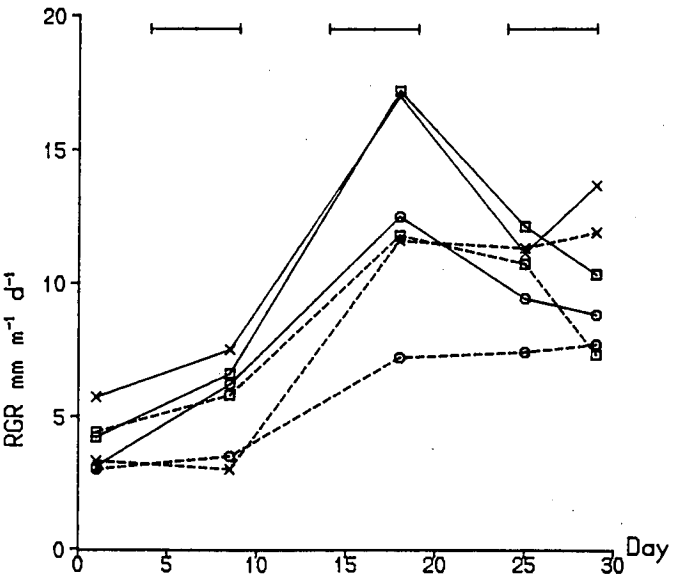


Fig. 7.1. Relative height growth rates of Sitka spruce seedlings, well-watered (solid lines) or exposed to drying cycles (dashed lines). Cross, nonmycorrhizal; circle, mycorrhizal with *Paxillus involutus*; square, mycorrhizal with *Thelephora terrestris*. Horizontal lines indicate the duration of the drying cycles.

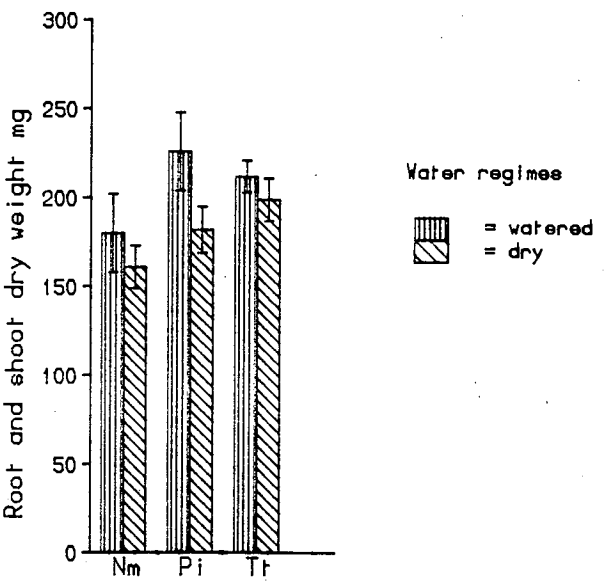


Fig. 7.2. Dry weights of Sitka spruce seedlings in Exp. 5. Nm = nonmycorrhizal, Pi = mycorrhizal with *Paxillus involutus*, Tt = mycorrhizal with *Thelephora terrestris*. Replicate numbers as in Table 7.1. Treatment means with two standard errors.

Table 7.1. Characteristics of nonmycorrhizal (NM) and mycorrhizal *(Paxillus involutus,* Pi and *Thelephora terrestris,* Tt) Sitka spruce seedlings, either well-watered (w) or exposed to drying and rewetting cycles (d), ± s.e.

	n	Mycorrhizas %	Root / shoot ratio
NM w	8	0.0±0.0	0.63±0.073
NM d	13	0.0±0.0	0.73±0.036
Pi w	8	69±8.7	0.53±0.027
Pi d	8	71±7.7	0.60±0.042
Tt w	22	73±5.7	0.60±0.022
Tt d	22	73±4.8	0.58±0.026

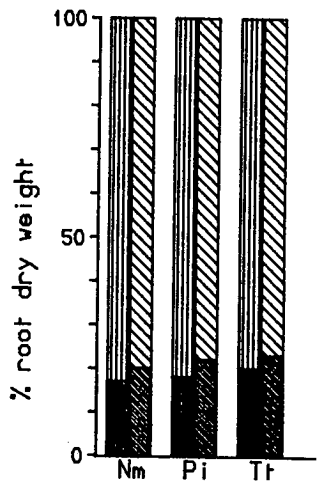


Fig. 7.3. Distribution of root dry weight between the upper (top of the column) and lower parts of the root systems in Exp. 5. Treatments and symbols as in Fig. 7.2.

Table 7.2. Distribution of roots in the pots of nonmycorrhizal (NM) and mycorrhizal *(Paxillus involutus,* Pi and *Thelephora terrestris,* Tt) Sitka spruce seedlings, either well-watered (w) or exposed to drying and rewetting cycles (d), ± s.e. and the corresponding ratio of root tip numbers / unit root dry weight. Top: upper 0-5 cm of the pot, Bottom: lower 5-10 cm of the pot, Total: mean of the whole root system.

Root dry weight mg

	Top	Bottom	Total
NM w	56±8.5	11±2.3	67±8
NM d	55±4.0	15±1.9	69±5
Pi w	63±7.2	16±4.2	79±9
Pi d	51±3.7	16±4.2	67±5
Tt w	64±3.7	16±1.7	79±4
Tt d	56±3.2	17±1.9	72±4

Root tips

	Top	Bottom	Total
NM w	163±24	22± 5.8	185±23
NM d	114±11	21± 4.9	139±15
Pi w	177±17	39±17.0	216±30
Pi d	101±16	24± 9.6	125±18
Tt w	175±13	27± 4.5	204±15
Tt d	170±18	28± 5.0	198±22

Root tips / mg root dry weight

	Top	Bottom	Total
NM w	3.02±0.40	2.11±0.63	2.91±0.42
NM d	2.06±0.16	1.29±0.22	1.90±0.16
Pi w	2.96±0.35	1.96±0.40	2.79±0.30
Pi d	2.08±0.40	1.35±0.20	1.91±0.30
Tt w	2.86±0.21	1.54±0.21	2.61±0.19
Tt d	2.97±0.21	1.53±0.17	2.64±0.18

The depth distribution of root dry weight was affected by drought only slightly (Table 7.2., Fig 7.3.), but more than that of root tip numbers (Table 7.2.). In droughted plants, slightly more of root dry weight was allocated below the top 5 cm of the pot than in controls, this effect being the same in all inoculation treatments. Total root tip numbers were decreased considerably by drought except in *Thelephora* mycorrhizal plants (Table 7.2.). The number of root tips per unit root dry weight was always lower at the lowest parts of the pot, and it was smaller in droughted nonmycorrhizal and *Paxillus* mycorrhizal plants than in watered controls, but similar in the droughted and watered *Thelephora* plants.

Shoot N, P and K concentrations were similar in the nonmycorrhizal and *Thelephora* mycorrhizal plants, being usually somewhat higher than those of *Paxillus* mycorrhizal plants regardless of watering treatment, which did not affect the nutrient concentrations much (Fig. 7.4.). The total contents of N, P and K were highest in *Thelephora* mycorrhizal plants in both water regimes, but this effect was most marked for P in the dry treatment: plants in the *Thelephora* treatment had twice as much P as plants in the *Paxillus* treatment, nonmycorrhizal being in between (Fig. 7.5.). This was mainly due to low P concentration in the *Paxillus* plants as their shoot dry weights were nearly as large as those of *Thelephora* plants. If the nutrient contents were different in the watered and droughted plants, they were lower in the dry treatment rather than higher, particularly so for the P content in shoots of *Paxillus* inoculated plants.

The growth and nutrient parameters were initially tested for the droughting effect by combining all inoculation treatments. The following variables were significantly reduced by the droughting treatment at $p<0.05$:

number of root tips in the whole root systems,
number of root tips per unit root dry weight in the whole root systems,
dry weight of the top part of the root systems,
and shoot nitrogen content;

at $p<0.01$, shoot potassium content.
As the level of significance required for multiple t-tests should be $p<0.001$, and this was not found in comparisons of individual means, the data are shown as treatment means with their standard errors in the tables and figures.

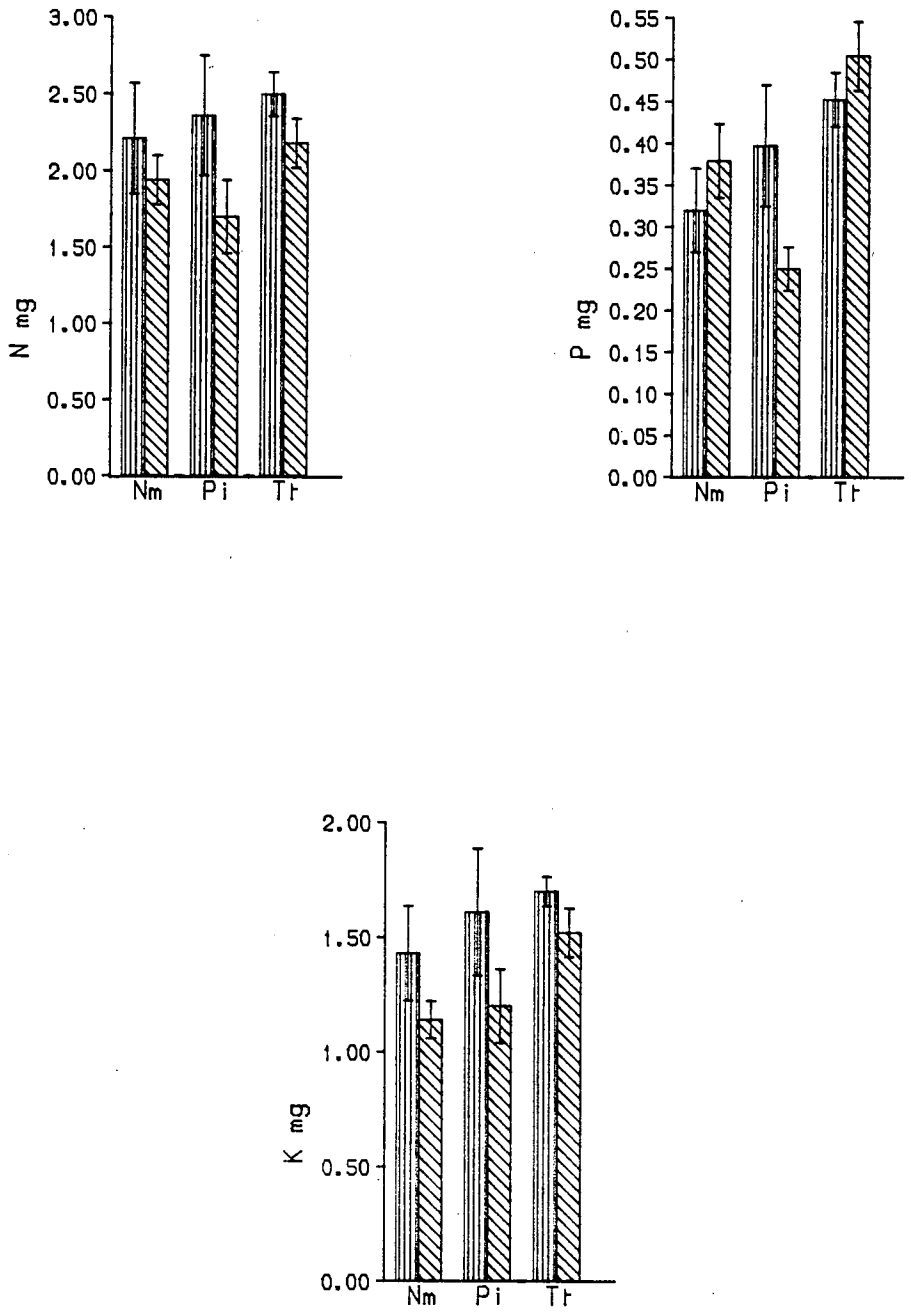


Fig. 7.4. Shoot nitrogen, phosphorus and potassium concentrations in Sitka spruce seedlings in Exp. 5. Treatments and symbols as in Fig. 7.2. Treatment means ± s.e.

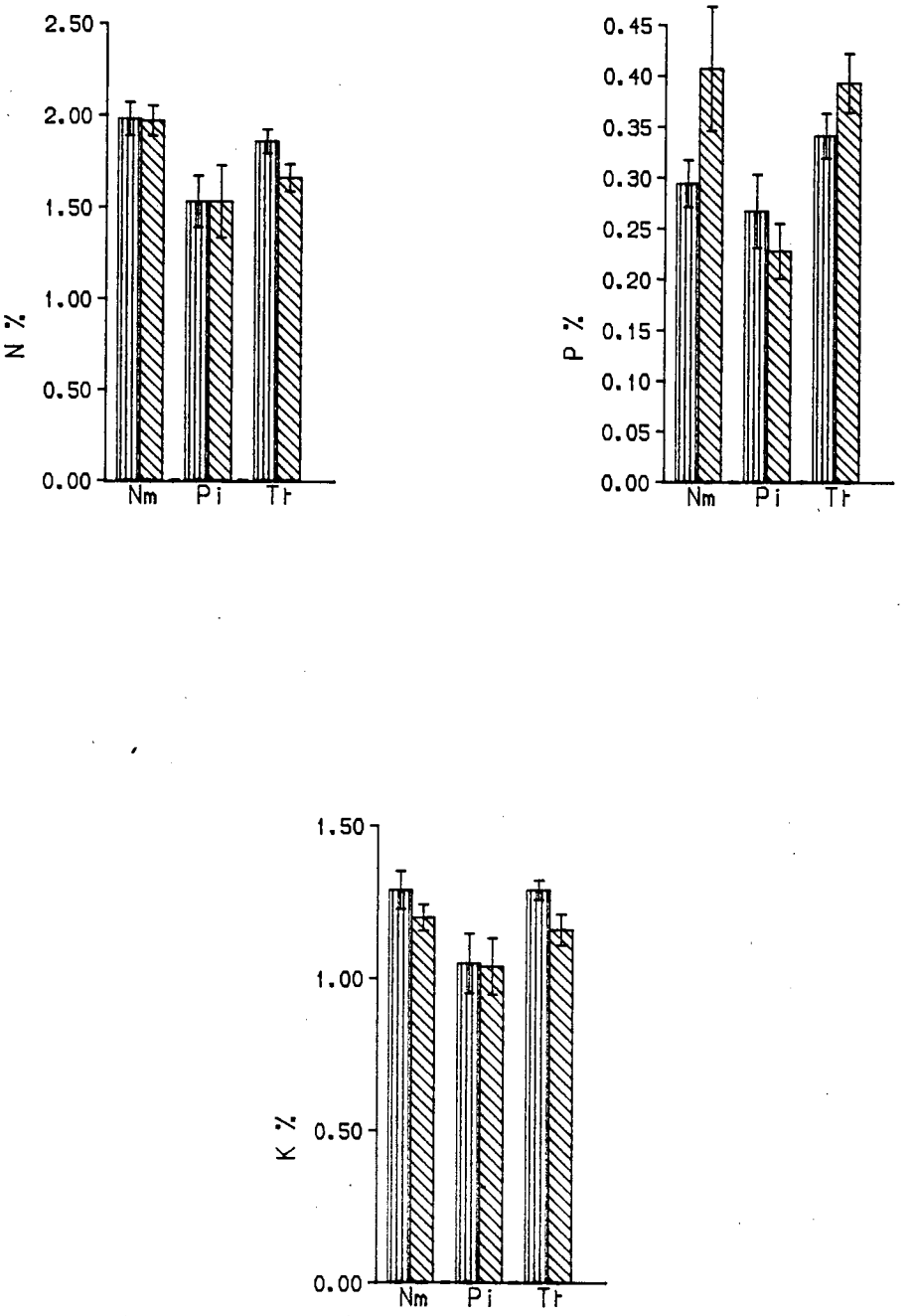


Fig. 7.5. Shoot nitrogen, phosphorus and potassium contents in Sitka spruce seedlings in Exp. 5. Treatments and symbols as in Fig. 7.2. Treatment means ± s.e.

The whole-plant transpiration rates measured from *Thelephora terrestris* mycorrhizal plants showed some day to day fluctuation, at least partly because the plants were growing during the period of transpiration measurements. The relative transpiration rate of droughted plants was lower than that of well-watered plants as soon as two days after the first watering, and remained so at least three days after rewatering, the difference becoming smaller (Fig. 7.6.).

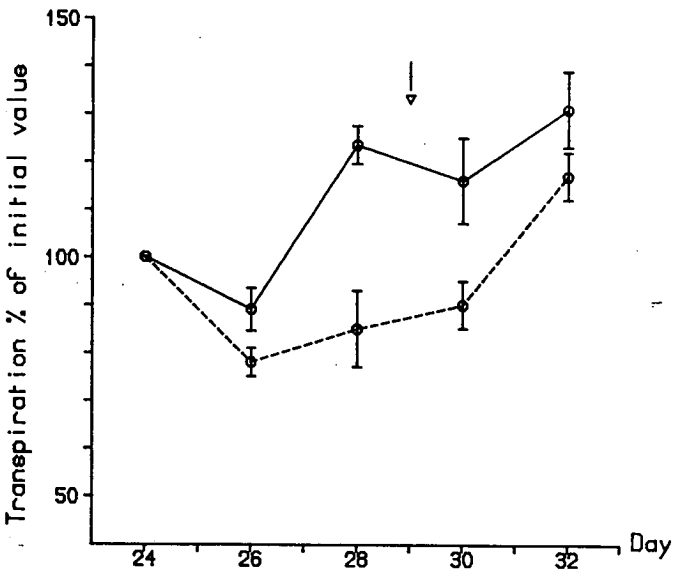


Fig. 7.6. Relative transpiration rates of well-watered (solid line) and drought treated (dashed line) Sitka spruce seedlings mycorrhizal with *Thelephora terrestris* in Exp. 5. Measurements done over the third drying cycle (last watering on day 23, rewatering on day 29 as indicated by arrow). Means of 11 replicate plants with two standard errors.

7.4 Discussion

As in earlier experiments, mycorrhizal effects on the reaction to droughting of adequately fed plants were small. However, many of the growth and nutrient parameters measured in this experiment were apparently unaffected by drought in *Thelephora* mycorrhizal plants, but affected in both the nonmycorrhizal and *Paxillus* mycorrhizal plants in a similar way. Therefore the difference between mycorrhizal fungi in these conditions seems to have been more important than any inherent difference between mycorrhizal and nonmycorrhizal plants.

Because of loss of replicate plants and consequently large standard errors, the comparison of treatments cannot be done with great confidence. The effect of repeated moderate drought treatment on total dry weight was rather small, even though the transpiration measurements done over the third drying cycle suggested that the stomata of the droughted plants were partially closed for a large part of the experiment. However, as found in earlier experiments (Chapters 4 and 5), moderate droughting of Sitka spruce seedlings may affect photosynthesis less than transpiration, and therefore the photosynthetic rates may not have been much affected. Nevertheless, the relative shoot growth rates were low in droughted plants, and the growth of root systems was reduced in the mycorrhizal treatments, similarly as found after the single drying cycle in Experiment 1. A small part of the reduction in shoot growth was due to increased root / shoot ratios in other inoculation treatments except *Thelephora terrestris*.

An increase in root / shoot ratios is a well-documented consequence of drought, but there is less information concerning growth allocation in different parts of the root system. In this experiment, a larger proportion of the root dry weight was allocated in the lower part of the root system in the plants exposed to drought. Even though this shift was small, it was consistent in all inoculation treatments, indicating preferential root growth in the lower part of the soil volume, which was hardly affected by the drought treatment. This effect could not have been caused by increased mortality of roots in the top, as no signs of dying of long roots were observed.

Similar effects were reported by Coutts (1982a) on Sitka spruce seedlings grown with their roots split between dry and moist soil. The growth of roots

was larger in the moist compartment, and the drought treated part of the divided root system was even smaller than a half of a root system with both parts in dry soil. In a field experiment, Feil *et al.* (1988) found that growth of Norway spruce *(Picea abies)* mycorrhizas was enhanced during drought particularly in mineral soil as opposed to the humus layer, which led to less superficial fine root distribution than in irrigated plots.

Root tip numbers (both absolute and relative to root dry weight) were decreased by the drying treatment in nonmycorrhizal and *Paxillus* mycorrhizal plants, but unaffected in *Thelephora* mycorrhizal plants. The decrease in root tip numbers relative to root dry weight was similar to that observed in Experiment 1 for the mycorrhizal *(Paxillus involutus)* treatment, even though the root tip numbers recovered within a week from rewatering. In that experiment, root tip numbers of nonmycorrhizal plants were unaffected by drought; however, they were always very low compared to those of mycorrhizal plants.

Other work has been reported, which shows a decline in fine roots of Sitka spruce in the field, but this has usually been attributed to increased mortality of fine roots rather than decreased root tip initiation and growth. Deans (1979) found that length of fine roots in a Sitka spruce stand on a peaty gley decreased at as high soil water potentials as -0.01 to -0.02 MPa, the critical soil water potential being slightly less negative in the open pored surface layer. However, this layer is more likely to be exposed to longer periods of low moisture, and the length of the dry period is most probably an important factor in determining root responses. Nisbet & Mullins (1986) compared fine root weights and tip numbers in three 22-25 year old Sitka spruce stands with differing drainage, and found a gradient in the amounts of dead and live roots, the mortality increasing with the likelihood of drying out of the top 0-10 cm layer of soil. However, Marshall (1986) did not find increased mortality in two-year-old Douglas fir seedlings unless they were also subjected to shading, and Feil *et al.* (1988) found an increase rather than a decrease in development of mycorrhizas in unirrigated Norway spruce both in trees in the field and in seedlings in pot experiments, whilst the growth of parent roots was suppressed. This led to increased branching density (number of lateral roots per unit length parent root) in the plants exposed to drought, which is contrary to the decrease in numbers of root tips per unit root weight in the present experiment, and in Exp. 1 (assuming root weight proportional to root length, which is not necessarily true). The increase in branching density of Norway

spruce occurred both in the humus layer (moder) and mineral soil (clay). These differences in the reactions of trees may be due to inherent species differences; the enhanced fine root growth reported for Norway spruce was not found in *Pinus sylvestris* in the same conditions (Bartsch 1985, unpublished dissertation, as quoted by Feil *et al.* 1988). However, factors such as duration of the drought, and carbohydrate status of the trees will affect the results too. Marshall (1986) suggested that depletion of carbohydrates is the primary reason for death of fine roots, rather than exposure to the dry soil environment as such. This same argument would apply to reduced growth of roots, such as observed in the present experiments.

The unaffected root tip numbers in *Thelephora terrestris* inoculated plants can be explained either as greater longevity of these mycorrhizas compared to nonmycorrhizal roots and *Paxillus involutus* mycorrhizas, or as sustained root tip initiation. Greater longevity would show as unaffected root tip numbers in the top of the root system, and sustained root tip initiation in the lower part. The variation in the data is rather too large to allow this question to be answered, but as hardly any dead root tips were found in the experiment, and as the root tip numbers of *Thelephora* mycorrhizal plants were unaffected by drought in each part of the root system, it would seem more likely that plants infected with this fungus were more able to grow new root tips than those in the other treatments.

The ecological significance of a growth pattern such as observed in the nonmycorrhizal plants, or growing long roots at the expense of new short roots, might be that rapid growth of long roots can extend the root systems to soil volumes which are still moist, whilst new root tips in dry soil are not very useful. Coutts (1981) showed that a few long roots extending to a moist lower compartment of split pots were enough to prevent the water potentials of Sitka spruce seedlings from decreasing, even though their stomatal conductances were lowered. On the other hand, summer rains may only wet the top part of the soil, and maintaining viable root tips in this layer could enable the tree to catch any available moisture before it evaporates (Deans 1979). Also, the topsoil tends to contain more available nutrients than lower parts of the profile.

Unaffected root tip numbers may have been the reason for *Thelephora* mycorrhizal plants having higher shoot phosphorus and also nitrogen contents than either nonmycorrhizal or *Paxillus* mycorrhizal plants in the dry treatment.

The P percent was as high in the nonmycorrhizal droughted plants, but as they had much smaller shoots due to growth allocation in root systems, their shoot P contents were small. Therefore the high concentrations were due to suppressed shoot growth rather than efficient P uptake in dry conditions. However, the droughted nonmycorrhizal plants performed better than droughted *Paxillus* mycorrhizal plants in terms of phosphorus uptake. The restricted P uptake of this fungus is somewhat surprising, as its ability to take up P was outstanding in vermiculite-peat in Experiment 1, and at least as good as that of nonmycorrhizal plants in perlite with nutrient solution in Experiments 2, 3a and 3b. Perhaps the repeated drying was particularly harmful for this fungus. It must also be noted that the substrate used here, perlite with soluble nutrients added, does not allow the full potential of mycorrhizal nutrient uptake to be manifested. It was used in order to diminish the mycorrhizal effect on nutrition in watered control plants, but it may similarly prevent mycorrhizal effects from showing in drought treated plants.

CHAPTER 8
EFFECT OF DROUGHT ON MYCORRHIZAL FORMATION BY DIFFERENT FUNGI

8.1 Introduction

In the previous chapters, experiments were reported on plants with either established mycorrhizas or no mycorrhizas at all, and a mycorrhizal effect on water stress avoidance was found when mycorrhizas also improved phosphorus uptake. However, from a practical point of view it may be as important to compare the initial stages of mycorrhizal formation in conditions of environmental stresses to which outplanted transplants are likely to be exposed. The existing roots of bare-rooted planting stock usually are mycorrhizal, but it may be the mycorrhizal formation in roots developing after outplanting that is important to the survival of the tree. In an optimal case, a nursery grown transplant or containerized seedling has an established mycorrhizal structure with a fungus which is able to grow and form new mycorrhizas and mycelial strands into the surrounding soil. But many fungal species common in nurseries do not thrive in a cold, dry, and often low nutrient soil in a typical outplanting site. The ectendomycorrhizal fungus is an extreme example of this: it has been found to be replaced by other mycorrhizal fungi soon after outplanting on most of the sites examined (Mikola 1965, Laiho 1967, Holden *et al.* 1983). Therefore it is necessary to test candidates for inoculation programmes in relation to stresses that recently planted seedlings need to survive.

The effects of drought and other environmental factors on mycorrhizal formation were discussed in Section 2.3.2. From the information available it appears that drought generally reduces colonization of root systems, but different fungal species react to water stress in a different way.

In this experiment, Exp. 6, the colonization of Sitka spruce roots by four fungal species which are considered useful for practical inoculation programmes (Wilson *et al.* 1987) was studied in different water regimes. Mycorrhizal formation was also related to the growth and nutrition of plants.

The fungi chosen were *Hebeloma crustuliniforme, Paxillus involutus, Laccaria*

proxima and *Thelephora terrestris*. Experience from field studies indicates that *Paxillus involutus* is common on adverse sites such as coal spoils and roadsides, whereas *Hebeloma crustuliniforme* and *Laccaria proxima* are often found in more fertile sites, typically brown earth in northern Britain (Ingleby, K., personal communication). *Thelephora terrestris* occurs in a wide range of habitats, even though Marx *et al.* (1984) have suggested it is particularly adapted to nutritionally and hydrologically good conditions prevailing in nurseries. These fungi also form different types of extraradical mycelium, ranging from the highly organized mycelial strands of *Paxillus involutus* and the slightly less differentiated strands of *Thelephora terrestris* (Agerer 1987) to single hyphae extending to soil from *Hebeloma crustuliniforme* and *Laccaria proxima* mycorrhizas.

8.2 Materials and methods

A completely randomized factorial block design was used with 4 fungi x 3 water regimes and 14 replicates in each of two harvests.

Sitka spruce seedlings were grown in 65 cm^3 'Erin' pots in vermiculite-peat mix (Section 3.1.) in a growth room with 20°C day / 15°C night temperatures, 16 h photoperiod with PAR ranging from 250 to 350 µmol $m^{-2}s^{-1}$, and 70 % relative humidity corresponding to a vapour pressure deficit of 0.71 kPa day, 0.51 kPa night.

Thirty-five days after germination plants were inoculated with a vermiculite-peat culture of one of *Hebeloma crustuliniforme, Paxillus involutus, Laccaria proxima* or *Thelephora terrestris*. The volume of the inoculum used for each plant was approximately similar, but it is possible that the different fungi had had different growth rates in the vermiculite-peat, which would have affected the density of the inoculum.

Before inoculation all pots were watered to saturation every other day with tap water, and the last two times, with Ingestad's solution (15 mg N l^{-1}). Differential watering started immediately after inoculation with the following water regimes:

1. saturated with distilled water every 2 days
2. saturated with distilled water every 7 days
3. saturated with distilled water every 14 days.

All plants were watered with 30 mg N l^{-1} Ingestad's solution every 14 days.

The first harvest took place 52 days after inoculation; alternate blocks were harvested. After the first harvest some of the plants of the driest treatment (3) were lost due to permanent wilting and over the final 31 days the watering treatments were altered to:

1. saturated with distilled water every 2 days
2. saturated with distilled water every 5 days
3. saturated with distilled water every 10 days,

with 50 mg N l^{-1} Ingestad's solution applied every 10 days to all treatments. After this alteration to the treatments the water loss from the pots over each drying cycle was approximately the same as at the beginning of the experiment, as estimated from gravimetric transpiration and evaporation measurements. Care was taken to saturate the pots especially in the dry treatments by applying water to them several times each day of watering. When nutrient solution was applied, this was done in several small doses too, after wetting the vermiculite–peat with water.

Due to the uneven radiation over the growth room bench, the water stress experienced by plants in different blocks was different. This was taken into account by rotating the blocks: at the watering of treatment 3, all blocks were moved by one fifth of the length of the bench. Compared to the variation in radiation with the length of the bench (or between blocks), the variation across the bench (or within each block) was small.

By the time of harvest 2 the plants were pot bound, and some replicates of other fungal treatments had been contaminated by *Thelephora terrestris* which had grown over the edges of the pots. Contaminated plants were left out of the analysis.

Eventually, the numbers of replicates in each treatment were:

	Hebeloma crustuliniforme			*Paxillus involutus*			*Laccaria proxima*			*Thelephora terrestris*		
	1	2	3	1	2	3	1	2	3	1	2	3
Harv. 1	12	14	14	13	14	14	14	14	14	14	14	14
Harv. 2	11	12	7	13	14	8	14	14	11	14	14	7

After each harvest numbers of root tips and mycorrhizal root tips were counted and root and shoot dry weights measured after drying in 85°C for 72 h. Nitrogen, phosphorus and potassium concentrations of shoots from the first harvest were determined.

The data were subjected to analysis of variance, and if that indicated significance, the least significant differences between main treatment means were calculated using Tukey's test at 0.05 and 0.01 levels (number of root tips, total dry weight, root / shoot ratio, root tip numbers / root dry weight ratio, total N, P and K). If the interaction of fungal and watering treatments was significant, all means of individual treatment combinations were compared at 0.05 significance level (mycorrhizal percentage and nutrient concentrations). Covariate analysis was used to remove the effect of varying proportions of mycorrhizal tips in the fungal treatments on nutrient concentrations and contents. This analysis was done separately in each of the three water regimes because of the effect of watering on mycorrhizal formation. The block effect was significant for many variables, and the block arrangement was therefore used throughout the calculations.

Correlation analysis was used to relate plant dry weights, shoot N, P, and K concentrations and contents to mycorrhizal percentages.

8.3 Results

8.3.1 Mycorrhizal formation

Colonization of the root systems by the mycorrhizal fungi was strongly affected by droughting, and the reaction of the different fungi varied (Fig. 8.1., Fig. 8.2.).

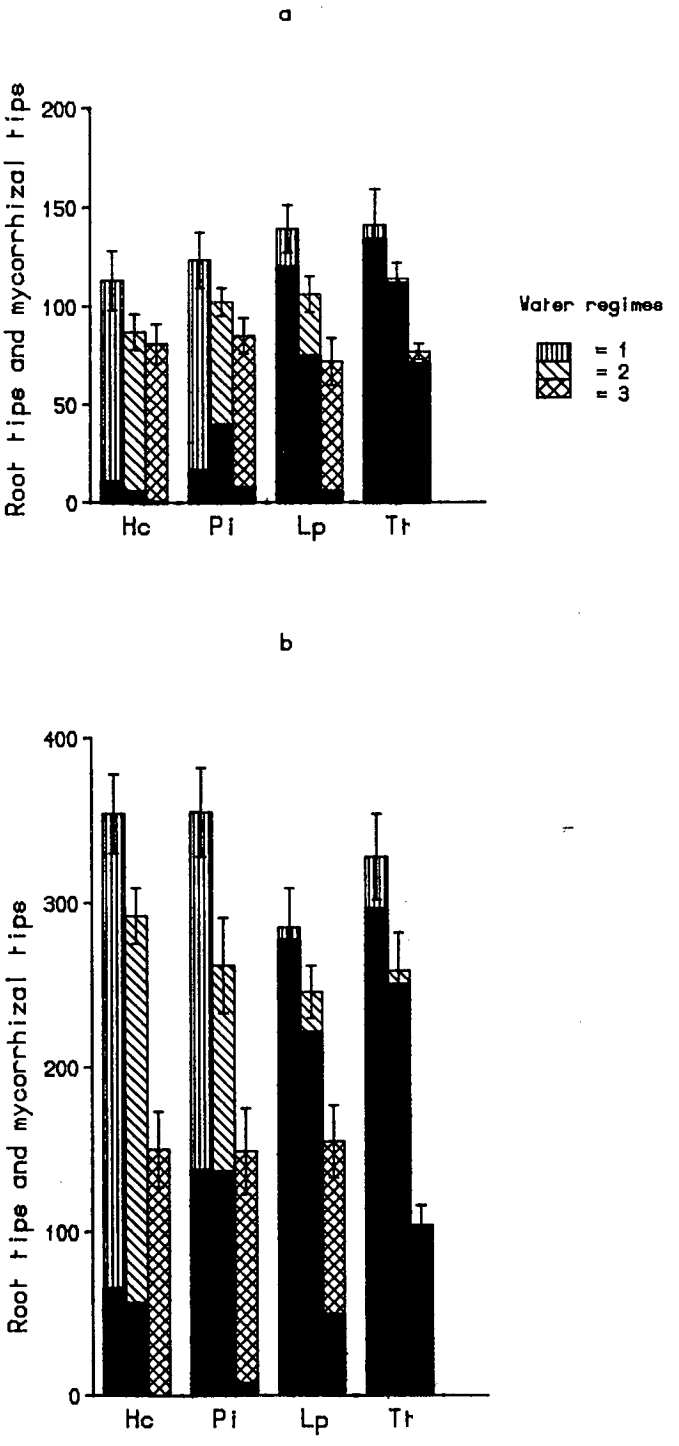


Fig. 8.1. Total numbers of root tips and mycorrhizal root tips (black) in Exp. 6. Errorbars refer to the total number of tips. Effect of fungal species, and interactions between fungal and watering treatments nonsignificant. a) Harvest 1, 52 days after inoculation, b) Harvest 2, 83 days after inoculation. Treatments: 1 = well-watered control, 2 = moderately droughted, 3 = severely droughted; Hc = *Hebeloma crustuliniforme,* Pi = *Paxillus involutus,* Lp = *Laccaria proxima,* Tt = *Thelephora terrestris*. All differences between watering treatments (1-2, 1-3, 2-3) significant ($p<0.01$., Tukey's test).

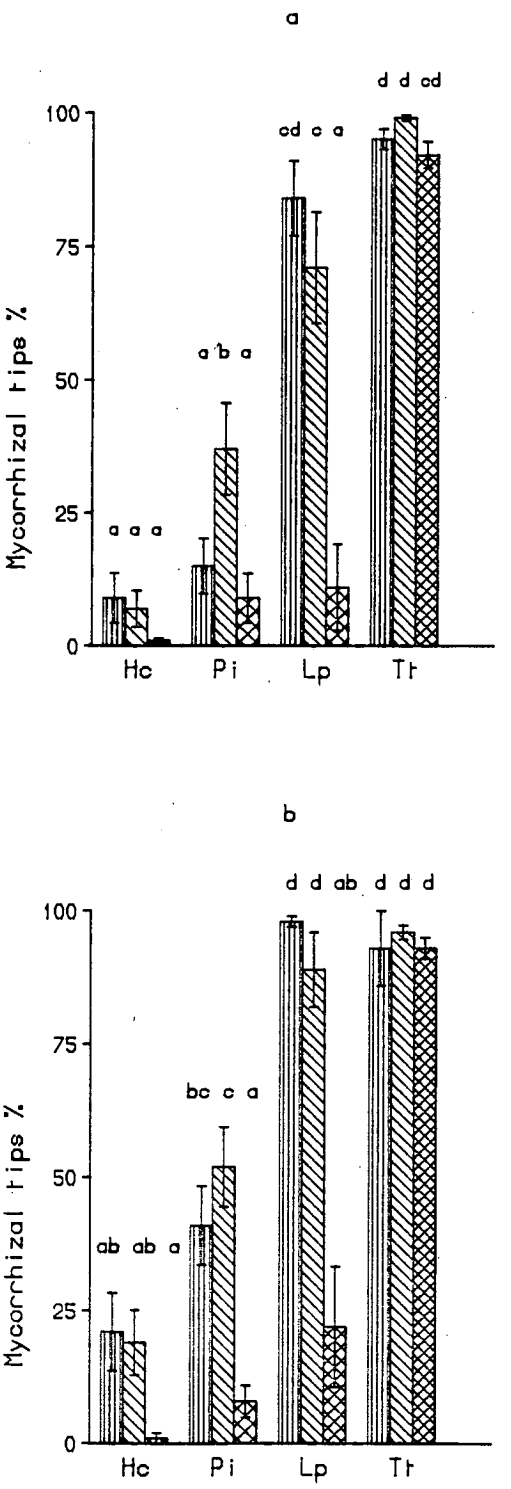


Fig. 8.2. Mycorrhizal root tips % of the total number of root tips with two standard errors in Exp. 6. Treatments and harvests as in Fig. 8.1. Two treatment means are significantly different ($P<0.05$) if not marked with the same letter.

Hebeloma crustuliniforme and *Paxillus involutus* were considerably slower in colonizing the root systems than were *Laccaria proxima* and *Thelephora terrestris*. *H. crustuliniforme* did not form more than 12 % mycorrhizal tips in the well-watered control treatment of the first harvest, and less than 1 % in the driest treatment. *L. proxima* formed 84 % mycorrhizal tips in the control, slightly fewer in treatment 2, but only 11 % in treatment 3. However, the mycorrhizal formation by *Thelephora* was largely unaffected by drought, more than 90 % root tips being mycorrhizal in each water regime. *P. involutus* performed significantly better in the intermediate water regime than either the control or severely droughted treatment, forming 37 % mycorrhizas.

At the second harvest, the differences between treatments had not changed much. Plants inoculated with *Laccária* and *Thelephora* retained their high mycorrhizal percentages in the control treatment, but *Laccaria* was still strongly affected by drought unlike *Thelephora*. The mycorrhizal proportion was not different between the driest treatment of *Hebeloma, Laccaria* and *Paxillus*. The controls of *Hebeloma* and *Paxillus* were catching up with the other fungi, and the difference between the water regimes 1 and 2 with *Paxillus* was diminished from the first harvest.

8.3.2 Growth of plants

Droughting significantly reduced both root and shoot growth of plants expressed as dry weights (Fig. 8.3., Plate 8.1.) or root tip numbers (Fig. 8.1). This effect was similar in all the fungal treatments at harvest 1, but at harvest 2, the mean dry weight of the *Paxillus involutus* treatment was significantly smaller than that of other fungal treatments in water regime 2. As indicated in Table 8.1., both total dry weights and root tip numbers in water regime 2 were about 80 % of those of the control plants, whereas in water regime 3 they were about 60 % in the first harvest and less than 50 % in the second harvest.

Plate 8.1. Sitka spruce seedlings in Exp. 6, water regimes: 1 = well-watered control, 2 = moderately droughted, 3 = severely droughted.

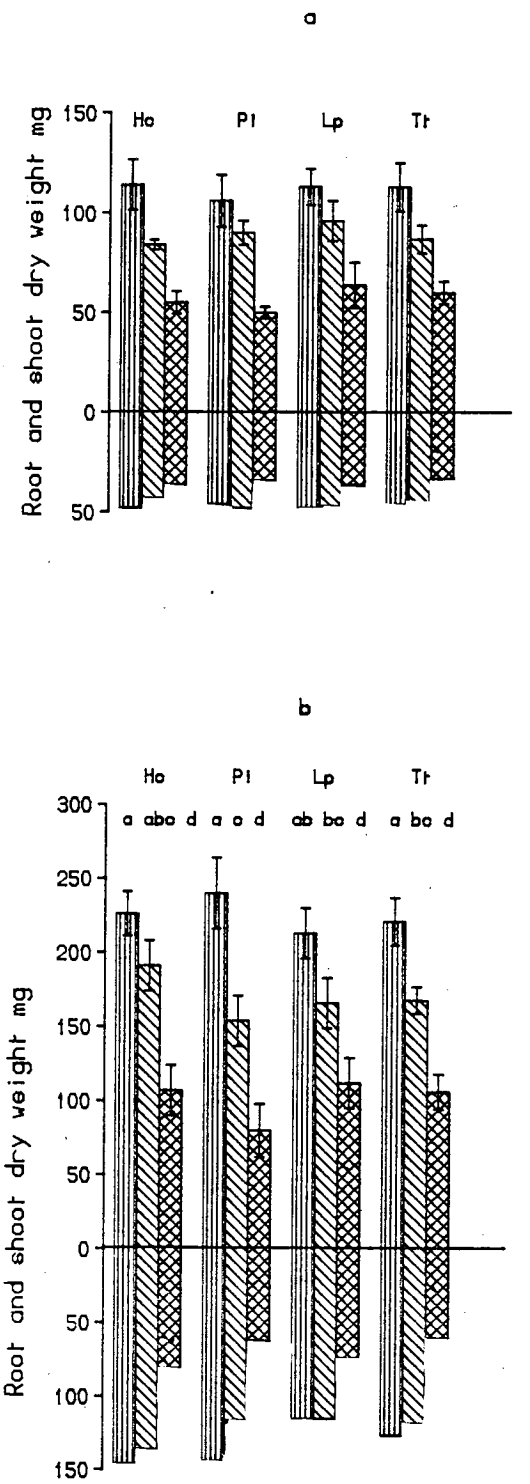


Fig. 8.3. Shoot and root dry weights in Exp. 6. Errorbars refer to the total dry weight. Treatments and harvests as in Fig. 8.1. Harvest 1: Effect of fungal species, and interactions between fungal and watering treatments nonsignificant. All differences between watering treatments (1-2, 1-3, 2-3) significant at 0.01 level (Tukey's test). Harvest 2: Two treatment means are significantly different at 0.05 level if not marked with the same letter.

Root / shoot ratios increased with the age of plants and, significantly, with increasing water stress (Table 8.2.). By the time of the second harvest, the differences between the two stress treatments had diminished somewhat. The fungal symbionts did not have different effects on root / shoot ratios at harvest 1, but at harvest 2 the differences between inoculation treatments were significant, *Paxillus* and *Hebeloma* having relatively more root dry weight than *Laccaria* and, particularly, *Thelephora* (Table 8.2.). The number of root tips per unit dry weight of root was decreased by droughting, and it was also significantly lower in plants inoculated with *Hebeloma* than those inoculated with *Thelephora,* other fungal treatments remaining in between at harvest 1 (Table 8.2.). At harvest 2, there were fewer root tips per unit root dry weight than before. This was probably due to the plants being pot bound, and growing unbranched long roots at the bottom of the pot.

Table 8.1. Total dry weights and root tip numbers of mycorrhizal plants in different watering treatments, expressed as % of control. 1 = watered control, 2 = moderate drought, 3 = severe drought; Hc = *Hebeloma crustuliniforme*, Pi = *Paxillus involutus*, Lp = *Laccaria proxima*, Tt = *Thelephora terrestris*.

Numbers of root tips

	Harvest 1			Harvest 2		
	1	2	3	1	2	3
Hc	100	77	72	100	82	42
Pi	100	83	69	100	74	42
Lp	100	81	55	100	79	32
Tt	100	76	52	100	86	54
Mean	100	79	62	100	80	43

Total dry weight

Hc	100	78	55	100	88	51
Pi	100	91	55	100	70	37
Lp	100	82	58	100	83	48
Tt	100	89	62	100	83	55
Mean	100	85	58	100	81	55

Table 3.2. Root / shoot ratios (a) and root tip number / root dry weight ratios (b) of mycorrhizal plants in different watering treatments, 1 = watered control, 2 = moderate drought, 3 = severe drought; Hc = *Hebeloma crustuliniforme*, Pi = *Paxillus involutus*, Lp = *Laccaria proxima*, Tt = *Thelephora terrestris*.

(a) Root / shoot dry weight ratio

	Harvest 1				Harvest 2			
	Water regime							
	1	2	3	Mean	1	2	3	Mean
Hc	0.43	0.51	0.64	0.53	0.66	0.72	0.76	0.71
Pi	0.44	0.54	0.70	0.56	0.62	0.79	0.76	0.72
Lp	0.52	0.51	0.61	0.55	0.59	0.70	0.68	0.66
Tt	0.42	0.53	0.57	0.51	0.58	0.72	0.57	0.62
Mean	0.45	0.52	0.63		0.61	0.73	0.69	

Significance of differences in root / shoot ratios between main treatments. * indicates significance at 0.05 level, ** at 0.01 level (Tukey's test).

	Treatment differences Mycorrhizal fungi						Water regime		
	Hc Pi	Hc Lp	Hc Tt	Pi Lp	Pi Tt	Lp Tt	1 2	1 3	2 3
Harv.1	ns	ns	ns	ns	ns	ns	**	**	**
Harv.2	ns	ns	**	*	**	ns	**	**	ns

(b) Root tip number / root dry weight ratio

	Harvest 1				Harvest 2			
	Water regime							
	1	2	3	Mean	1	2	3	Mean
Hc	2.27	2.07	2.22	2.19	2.43	2.20	1.91	2.18
Pi	2.70	2.20	2.54	2.48	2.51	2.24	2.30	2.35
Lp	2.91	2.31	2.06	2.43	2.29	2.20	2.06	2.18
Tt	3.02	2.61	2.42	2.68	2.61	2.18	1.87	2.22
Mean	2.73	2.30	2.31		2.46	2.21	2.04	

Significance of differences in root tip number / root dry weight ratios.

	Treatment differences Mycorrhizal fungi						Water regime		
	Hc Pi	Hc Lp	Hc Tt	Pi Lp	Pi Tt	Lp Tt	1 2	1 3	2 3
Harv.1	ns	ns	*	ns	ns	ns	**	**	ns
Harv.2	ns	ns	ns	ns	ns	ns	ns	**	ns

8.3.3 Nutrition

The nitrogen, phosphorus and potassium concentrations of the plants were significantly different in different fungal treatments (Fig. 8.4., Table 8.3.), *Thelephora* plants having higher P and K concentrations than any other fungal treatment. *Hebeloma* plants had lower N concentrations than *Paxillus* or *Thelephora* plants, and lower P than any other fungal treatment. N and P concentrations were higher in the water stressed plants than in controls. P concentration also showed an interaction between mycorrhizal and watering treatments, due to the high concentration in *Thelephora terrestris* mycorrhizal plants in water regime 3.

Total nutrient contents in the shoots showed the same declining trend with increasing water stress as total dry weights did (Fig. 8.5, Table 8.3), but now fungal effects were significant as well as watering effects. *Thelephora* inoculated plants contained more N than *Hebeloma* plants, total P was higher in *Thelephora* than any other fungal treatment, and higher in *Laccaria* than *Hebeloma* mycorrhizal plants. The K content of *Thelephora* plants was higher than that of plants inoculated with *Paxillus* or *Hebeloma*.

When the variation in the mycorrhizal percentages was taken into account by using it (angle transformed) as a covariate in analysis of variance, separately in each water regime, the differences in N, P, and K concentrations or contents between the fungal treatments were no longer significant, except for the P % in water regime 1 (with unadjusted data, this was significant in each water regime). The ranking order of the adjusted means of N, P and K concentrations and contents remained the same in covariate analysis as in analysis of variance (adjusted data not shown).

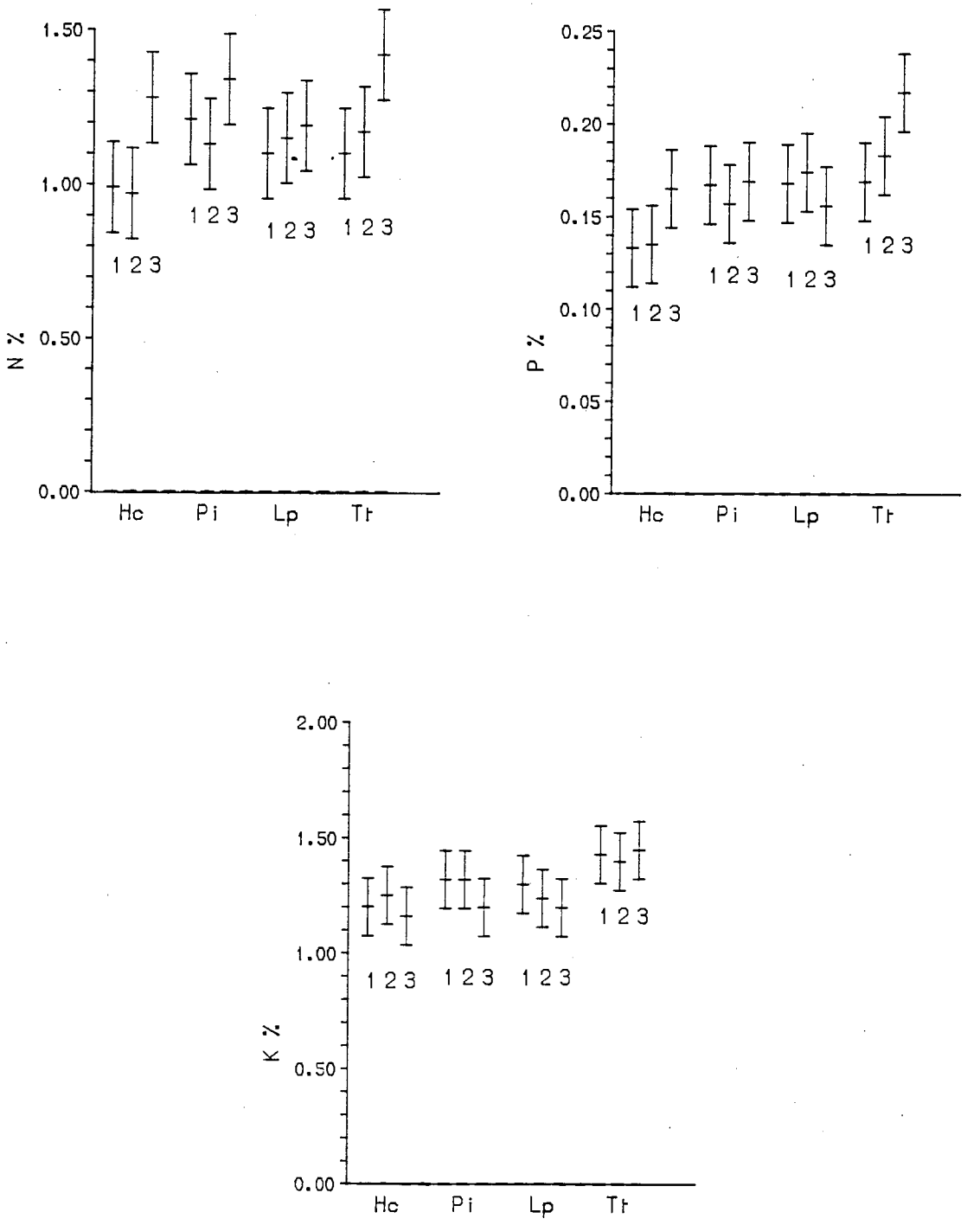


Fig. 8.4. Nitrogen, phosphorus and potassium concentrations in Sitka spruce shoots, % of dry weight in Exp. 6 at Harvest 1, 52 days after inoculation. Treatments as in Fig. 8.1. Vertical bars indicate least significant difference between individual means at 0.05 level (Tukey's test).

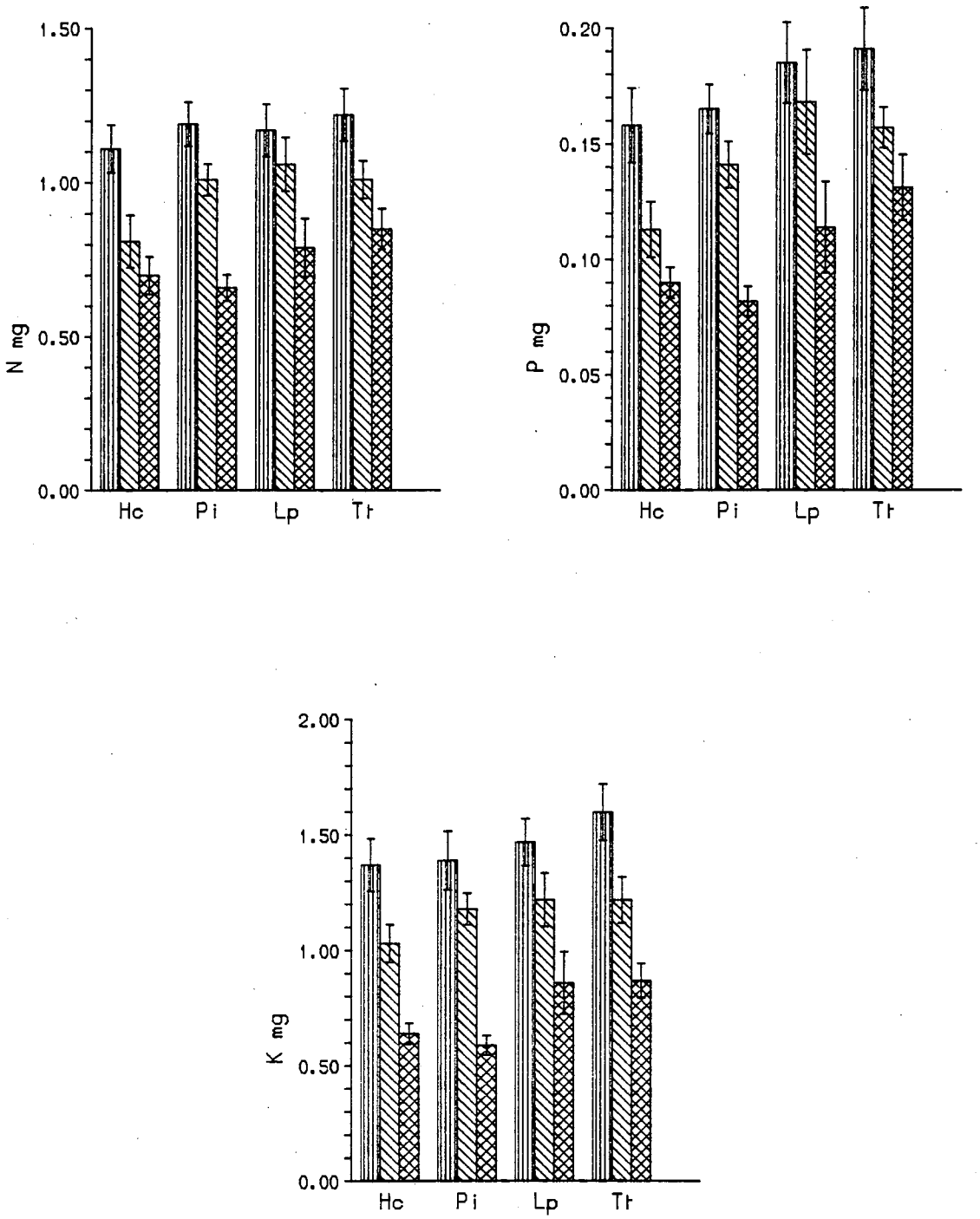


Fig. 8.5. Nitrogen, phosphorus and potassium contents in Sitka spruce shoots, mg, means ± s.e. in Exp. 6 at Harvest 1, 52 days after inoculation. Treatments as in Fig. 8.1. Significance of differences indicated in Table 8.3.

Table 8.3. Significance of differences in shoot N, P, and K concentrations and contents between main treatments. Because of a significant interaction, P % is not shown; compare to Fig. 8.5. and Fig. 8.6. Hc = *Hebeloma crustuliniforme*, Pi = *Paxillus involutus*, Lp = *Laccaria proxima*, Tt = *Thelephora terrestris*. 1 = watered control, 2 = moderate drought, 3 = severe drought. * indicates significance at 0.05 level, ** at 0.01 level (Tukey's test).

	Treatment differences Mycorrhizal fungi						Water regimes		
	Hc Pi	Hc Lp	Hc Tt	Pi Lp	Pi Tt	Lp Tt	1 2	1 3	2 3
N %	*	ns	*	ns	ns	ns	ns	**	**
K %	ns	ns	**	ns	**	**	ns	ns	ns
Tot. N	ns	*	*	ns	ns	ns	**	**	**
Tot. P	ns	**	**	ns	**	**	**	**	**
Tot. K	ns	ns	**	ns	*	ns	**	**	**

8.3.4 Correlation analysis

The correlation coefficients between total dry weights and N and P concentrations were negative in most cases (coefficients not shown). Total dry weight correlated significantly with mycorrhiza percentage only in two cases, in water regime 2 with *Paxillus* and in water regime 3 with *Laccaria*. Both fungi had a correlation of mycorrhizal percentages with total phosphorus content and phosphorus concentration of the shoots too (Table 8.4). The range of the extent of infection was very narrow in both *Thelephora* and *Hebeloma,* which explains the fact that their mycorrhizal percentage did not show a correlation to dry weight or nutrients. When all fungal treatments were combined, all correlation coefficients between mycorrhizal percent and nutrient contents in water regimes 2 and 3 were significant and positive, even though not very high.

Table 8.4. Correlation coefficients of mycorrhizal percent [(angle)^3] with total dry weight (dwt) and shoot N, P, and K concentrations and contents of Sitka spruce seedlings. * indicates significance at 0.05 level, ** at 0.01 level. See text for treatments.

Treatments		Dwt	N %	P %	K %	Total N	Total P	Total K
Hc	1	.19	.26	-.27	.13	.45	-.04	.20
	2	.07	-.37	-.48	.13	-.29	-.28	.13
	3	-.34	.07	-.01	.29	-.20	-.27	-.14
Pi	1	.35	-.25	-.30	-.37	.18	.09	.12
	2	.66*	.34	.66*	.41	.64*	.75**	.63*
	3	.38	.05	-.06	.25	.21	.09	.39
Lp	1	.39	-.31	.16	-.17	.26	.46	.34
	2	.18	.10	.65*	.45	.31	.54*	.40
	3	.79**	-.29	.42	-.08	.45	.75**	.57*
Tt	1	.26	-.08	.34	-.43	.33	.38	.24
	2	.17	.35	-.22	-.19	.48	.12	.10
	3	.27	.39	.52	.21	.38	.45	.32
All	1	.16	-.09	.18	.20	.20	.37**	.28
	2	.12	.30*	.56**	.24	.32*	.43**	.30*
	3	.10	.31*	.61**	.51**	.32*	.49**	.35*

8.4 Discussion

8.4.1 Mycorrhizal formation

The differences between the four mycorrhizal fungi in their ability to form mycorrhiza in different water regimes proved considerable. *Hebeloma crustuliniforme* and *Paxillus involutus* were slower colonizers than the other two fungi, and therefore the initial stages of infection cannot be compared. The differences in the speed of colonization may have been due to the density of fungal hyphae in the inoculum. However, if this was smaller with some fungi than others, it was probably a result from slow growth in culture, resulting from an inherent difference in the growth rates of the fungi.

Paxillus showed preference to moderately droughted conditions especially in the early phase. *Thelephora terrestris* was rather indifferent to water regime, and both *Laccaria proxima* and *Hebeloma crustuliniforme* formed mycorrhizas more efficiently in conditions of abundant watering.

This result reflects partly what is known of the ecology of the fungi. *Laccaria proxima* is adapted to fertile sites with plenty of available water, as is *Hebeloma crustuliniforme*. Moreover, *H. crustuliniforme* was classified as drought intolerant by Coleman *et al.* (1989) in a pure culture study. *Paxillus involutus* strains have been found on very adverse sites such as mine spoils where they are exposed to drought and other environmental stresses such as low nutrient availability and high heavy metal concentrations (Denny & Wilkins 1987). Both *Laccaria* and *Thelephora* are efficient early stage colonizers, and common in forest tree nurseries. In field experiments *Thelephora* has proved as good an inoculant as other fungi tested in northern Britain, and extensive natural colonization by this fungus showed that it was well adapted to the conditions on afforestation sites (Wilson *et al.* 1987). Thomas *et al.* (1983) also found *Thelephora terrestris* in a range of outplanting sites, and therefore considered it as a useful species for inoculation purposes.

However, it is a commonly held view that *Thelephora* is a nursery fungus not particularly adaptable to field situations (Marx *et al.* 1984). This is largely based on results showing improved performance of forest tree seedlings mycorrhizal with *Pisolithus tinctorius* compared to *Thelephora terrestris* in the

southeast of the U.S.A., where the climatic conditions are different from those in northern Britain. In particular, *P. tinctorius* is tolerant to high soil temperatures (Marx *et al.* 1970), which may be an explanation of its influence on plants in warmer climates.

Another reason for these different views concerning *Thelephora terrestris* may be different behaviour of different strains of the fungus; Holden *et al.* (1983) showed that a strain of this fungus isolated from a forest site outperformed a nursery isolate in experiments in forest soil. In the present experiment, only one strain of each fungus was used, and the results might have been different with other isolates.

The four species studied form four different types of extraradical mycelium. *Paxillus* grows highly organized mycelial strands with large vessel hyphae surrounded by smaller structural ones, with abundant hyphae radiating from the surface of the strands, which are a distinctive feature of *P. involutus* strands as opposed to many other fungi (Fox 1983). *Thelephora* has a strand structure too, even though the degree of organization of the hyphae varies. *Laccaria proxima* has no strand structure and there is little mycelium extending to the soil. *Hebeloma* does form external mycelium but it is not organized to strands but grows as a white woolly web. Mycelial strands have been shown to take up and translocate water at similar rates as roots of plants (Duddridge *et al.* 1980), and they also function in the spreading of the fungus from root to root (Fox 1983, Read *et al.* 1985). However, if strand formation is of particular advantage in sites susceptible to temporary drought, as suggested by Parke *et al.* (1983), the strand forming fungus *P. involutus* might have been expected to perform better in dry conditions than the other fungi. This it did in the intermediate water regime 2, but in the driest conditions it formed few mycorrhizas.

As outplanted seedlings are likely to be exposed to at least temporary water stress in a wide range of sites, the differences between mycorrhizal fungi should be taken into account in this respect. *Thelephora terrestris* and *Paxillus involutus* seem to be promising fungi for afforestation of adverse sites liable to drying out, but the slowness of *P. involutus* as a colonizer in comparison to *T. terrestris* may reduce its usefulness in inoculation programmes, as may the fact that the mycorrhizal formation by this fungus was associated with reduced growth of plants in one water regime. On the other hand, use of such fungi as

Laccaria proxima and *Hebeloma crustuliniforme* should be concentrated on sites not liable to drying out.

8.4.2 Growth and nutrition of plants

The observed differences in mycorrhizal formation between the different species were not reflected in the survival rates or the size of the seedlings; more plants had died due to wilting in the *Thelephora terrestris* treatment than in *Paxillus involutus* or *Laccaria proxima* treatments by the time of the second harvest, and root tip numbers and plant dry weights decreased with increasing moisture stress in the same way in all fungal treatments. The only exception of this trend was that the growth of the plants with *Paxillus involutus* was more adversely affected by the moderately droughted treatment than was plant growth in the other fungal treatments at the second harvest, although the more numerous mycorrhizas formed by this fungus in this water regime compared to the watered control had suggested good drought resistance at the first harvest. Hence relatively efficient colonization by a fungus in some environmental conditions cannot always be regarded as a sign of improved performance of the host plant.

The allocation of growth was affected by the fungus used, although this effect was not consistent. The root / shoot ratios, and the number of root tips per unit root dry weight were decreased by drought in the same way as in previous experiments (Chapter 4, Chapter 7), and *Thelephora terrestris* mycorrhizal plants had remarkably low root / shoot ratios in the severely droughted treatment. Moreover, the plants heavily mycorrhizal with *Thelephora terrestris* had relatively more root tips than plants with other fungi, particularly *Hebeloma crustuliniforme*. As suggested in the context of Experiment 5, the slightly different allocation of growth of plants mycorrhizal with *Thelephora terrestris* may have been related to their improved nutrient uptake, although the overall growth was not significantly affected.

N and P concentrations of shoots were highest in water stressed plants, which were considerably smaller than well-watered ones, but total nutrient contents in shoots were always lower in stressed plants. The accumulation of N and P in slowly growing plant shoots shows that inhibition of uptake of these nutrients from dry soil was not one of the main reasons for the slow growth of

water stressed plants. Viets (1972) suggested that root systems subjected to cyclic drought may be very efficient in taking up and storing nutrients during relatively short periods of adequate soil moisture, and consequently, available nutrients. Mycorrhizal structure may be expected to be particularly helpful in a situation like this, as mycelium is known to store nitrogen (Alexander 1983), and phosphorus as polyphosphate granules (Harley & Smith 1983), and it may also recover from drought relatively quickly. The higher correlations of mycorrhizal percentages with plant nutrient concentrations and contents in the two water stress treatments compared to the control suggest that the mycorrhizal structure was more important in nutrient uptake during drying cycles than in conditions with adequate water.

A comparison of the nutrient concentrations and contents of the seedlings with varying mycorrhizal proportions indicates a benefit from a high degree of mycorrhizal colonization both in watered and dry conditions. In particular, the phosphorus uptake by *Thelephora terrestris* was very high compared to the effectively nonmycorrhizal roots of plants inoculated with *Hebeloma crustuliniforme*. Moreover, when the results were adjusted for varying mycorrhizal proportions, most of the differences between the fungal species were rendered nonsignificant. Hence the differences between these strains of the four fungi were, in the main, meaningful in terms of their efficiency in colonizing the root systems rather than in terms of their effect on growth and nutrition of plants.

CHAPTER 9
GENERAL DISCUSSION

"What we observe is not nature itself, but nature exposed to our method of questioning."

Werner Heisenberg

9.1 Evaluation of experimental approaches and methods

9.1.1 Use of mycorrhizal and nonmycorrhizal seedlings

In this section, some of the problems encountered are discussed, as well as possible ways of overcoming them in future work. In the subsequent sections the results are evaluated and their implications for the understanding of the ectomycorrhizal habit on the one hand and for practical silviculture on the other hand are discussed, and proposals for further research are pointed out.

In most experiments reported here, the reactions of very young, mycorrhizal and nonmycorrhizal seedlings were studied. Use of young seedlings is a common approach in studies into tree physiology, and, for practical reasons, the only one possible if mycorrhizal and nonmycorrhizal seedlings are to be compared. Use of nonmycorrhizal plants in experiments has been criticized because nonmycorrhizal roots are inherently different from mycorrhizal, and plants without mycorrhizas occur only as artefacts. However, it is this 'inherent difference' between mycorrhizal and nonmycorrhizal roots that has been extremely useful in demonstrating the importance of the mycorrhizal habit in plant nutrition and survival in field conditions as well as the function of mycorrhizas in resistance to plant pathogens. Similarly, it can be expected to clarify the role of mycorrhizas in plant water relations.

The use of semiaseptic conditions necessitated use of very young plants in experiments, and yet contamination by airborne spores mainly of *Thelephora terrestris* was found in all experiments except Experiment 6. Plants in this experiment were the only ones that were kept in a growth room from sowing to harvest. All the other seedlings were in a glasshouse for at least some

weeks, which apparently was the reason for their early contamination. Lack of space is a limitation to the number of plants and experiments which can be run in controlled environments, but access to such rooms appears to be a necessity in these types of experiments. Perhaps the time of the year is a factor affecting contamination, and it would be expected that there are few spores in the air in a cold winter.

Another problem involved in the production of mycorrhizal and nonmycorrhizal plants is the large influence of mycorrhizas on nutrient uptake from soil, and the fact that high nutrient levels inhibit mycorrhizal formation. Therefore the use of an appropriate nutrient regime is of crucial importance in mycorrhizal work. In these experiments, it proved necessary to find a way of controlling the nutrition of plants to be able to compare the water relations of seedlings without the interference of different nutrient status and size. However, in soil and also in an artificial mixture containing peat such as vermiculite-peat this is nearly impossible, if moderate fertilization is used, as the differences between nutrient uptake of mycorrhizal and nonmycorrhizal plants are largest at low soil N and P levels. Low nutrient regimes, then, are desirable because high nutrient concentrations in the substrate are known to inhibit mycorrhizal formation. Use of a completely artificial system, perlite with nutrient solution, proved to be the best way in which appropriate plants could be produced; although the mycorrhizal plants still tended to have higher nutrient concentrations than nonmycorrhizal, they were comparable, and their size was similar (Experiment 3a). The low fertilizer regime used during the early growth of the seedlings ensured intense mycorrhizal colonization. However, the same ability of mycorrhizas to take up nutrients from soil, which are inaccessible to nonmycorrhizal roots, may affect the ecological relevance of results from such studies, as discussed in Section 9.2.

9.1.2 Pot experiments in water relations studies

A large part of the extensive information on water relations of plants and their reactions to water stress has been derived from pot experiments in which plants are subjected to one drying cycle, in the same way as in Experiments 1 and 3. This approach is useful in that it enables the control of environmental factors, but it has its limitations. Growing plants in pots often leads to pot bound conditions, and therefore allows the drought treatment to dry the soil

unrealistically rapidly as a result of high rooting densities. Hence the time course of events may be affected, even though this is counteracted by the lack of competition by other plants in pots. Therefore the choice of pot volume and time of commencing experiments (in terms of plant size) is important, as it involves balancing a large rooting volume and very slow induction of drought, and a too small rooting volume.

The use of relatively narrow and deep pots in Experiment 1 (Chapter 4) ensured that the root systems did not become pot bound. However, as the contents of a deep pot dry out gradually as a result of evaporation and transpiration, a moisture gradient inevitably forms with the depth of the pot, the top soil being driest. In this experiment, the larger root systems of mycorrhizal plants as well as the occurrence of mycelial strands accounted for the fact that this gradient was not similar in the pots of mycorrhizal and nonmycorrhizal plants at the time of the measurements. Because a substantial quantity of moisture remained in the lower parts of the deep pots, particularly in those of the smaller nonmycorrhizal plants, the root environment of the different plants was not similar. Moreover, the time required for the drying of the soil enough to cause measurable stress was long enough for significant differences in the growth and nutrition of the plants to be manifested. Even though this yielded some interesting results as such, the physiological measurements were not easy to interpret on this basis.

One possible way of evaluating the importance of a moisture gradient and, at the same time, providing an estimate of the soil water status would have been to measure soil water potentials at different depths in the pot. Lopushinsky & Klock (1974) estimated mean soil water potentials in pots of coniferous seedlings subjected to drying, by measuring soil water potentials with psychrometers in two locations. The two readings from the same depth in the pots in an area of high root concentration differed by less than 0.2 MPa in 66 % of measurements. However, an inaccuracy of 0.2 MPa in soil water potential measurements brings about doubts concerning the meaningfulness of the measurement, as this may be caused by uneven distribution of water in pots rather than lack of precision of measurement.

Therefore another way of diminishing the significance of gradients was chosen: this involved use of smaller pots in which the root systems were nearly pot bound at the time of the measurements, and also use of perlite as a substrate

with lower water retention capacity (Experiment 3, Chapter 5). Therefore the substrate dried out more quickly around the roots, and even though there was a slight difference in the growth of droughted and watered plants, this was not yet significant at the time of the measurements. Even though there was a moisture gradient in these pots, this must have been less steep because of the small size and denser rooting. Measuring the moisture content of the whole pot rather than the water potential at some point - necessarily arbitrary - was the way of integrating the small-scale variation in the moisture; in Experiment 1 this was not a particularly good measure for the environment of the root systems, as the roots did not always extend to the bottom of the pots, but in Experiment 3 in which smaller pots were used, this was probably the most reliable measure for the moisture in soil. Experiment 3a was the most successful attempt to do this in the series of the short-term experiments in this work. In this experiment, plant water potentials were measured as the plants became progressively more water stressed, and this was expressed as water potential against soil moisture. Thus the differences in soil moisture content in the pots of different plants were used for advantage whereas in Experiment 1 and Experiment 3b this caused undesirable variation in the results, as the size of the plants and their root / shoot ratios varied somewhat, and therefore the plants were at varying phases of water stress at the fixed time of measurements. The extent of this variation between plants was unexpectedly large, especially in Experiment 3b.

Experiments 5 and 6 (Chapters 7 and 8) involved an attempt to accommodate the spatial and temporal variation in soil moisture, and to study the effects of this on plant growth and nutrition, rather than to eliminate it. Growth analysis of droughted plants in pot experiments was criticized by Sands & Rutter (1959) because limited rooting volumes will affect the results. In the present work, terminating the experiments early enough for the plants not to be pot bound (with the exception of the second harvest in Experiment 6) diminished the risk of the results becoming confounded by limiting rooting space. Nevertheless, this possibility cannot be totally ignored.

9.1.3 Gas exchange

Two different, open systems were used for measuring gas exchange in Experiments 1 and 3, respectively, a laboratory system with controlled air

humidity, and a portable system. In the laboratory system, steady-state measurements of CO_2 and H_2O exchange were obtained after 15-20 minutes equilibration. The advantage of this was a reliable measurement of gas exchange, but the long time period required for each replicate group was a disadvantage. Therefore measurements were carried out over a large part of the day, and even though they did not commence until 6 h after the onset of the light period, this produced some variation in the results as shown in the following measurements of the stomatal conductance of one group of plants on the same day.

Time	g_s mmol $m^{-2}s^1$
11.00	113
14.30	112
18.00	108

This variation was somewhat larger than reported by Watts & Neilson (1978) for Sitka spruce seedlings in controlled environments. In their experiments, most of the changes in photosynthesis, stomatal conductance and water potential occurred within 3 h from switching on the lights, and equilibrium values were reached within 6 h.

Use of the portable system involved leading dry air into the leaf chamber, and therefore the measurement was completed within 60 s from enclosing the seedling to ensure that the stomata did not have time to react to the new conditions. This type of measurement tends to lead to large variation in the results, as it measures the functioning of the plant without equilibration, even if care is taken to avoid changes in radiation and temperature around the plant, and to keep the CO_2 level in the growth room constant.

The choice of the method obviously depends on the availability of equipment, but also on the experimental approach. In these types of experiments, in which a point measurement of gas exchange parameters was all that was required, the quick method is probably more advantageous. It enables gas exchange determination of several replicates at the same time of the day, and other measurements (here plant water potential and soil moisture) can be done immediately after finishing the gas exchange measurements. If daily trends or, for instance, information on stomatal sensitivity to rapid changes in the environment are required, a more controlled system is needed.

9.2 Physiological and ecological implications of the results

9.2.1 Water relations of mycorrhizal and nonmycorrhizal seedlings

The first hypothesis propounded in this work was that mycorrhizal structure increases the short-term drought resistance of Sitka spruce seedlings independently of nutrient effects. When nutrition of the experimental plants was rigorously controlled, this hypothesis was not supported, but the whole-plant conductance to water was inferred to be lower rather than higher in mycorrhizal plants as opposed to nonmycorrhizal, and their CO_2 assimilation rates were not higher, either. As discussed in Chapter 5, the difference may have been in the root conductance of the seedlings.

The low conductance of roots compared to other parts of the pathway of water within plants is largely due to the fact that water has to cross the membrane to enter the plant symplast. It is not known whether the major pathway of water in the cortex of (non-ectomycorrhizal) roots is through the symplast or through the apoplast. If it is through the apoplast, this means that the soil solution can move as far as the endodermis (Kramer 1983). In ectomycorrhizal roots, one of the possible pathways is in the interhyphal and intercellular spaces of the cortex as well (Reid 1979), the conductance of which depends on the conductance of the fungal and host cell wall. The pathway of water in mycelial strands was considered apoplastic by Duddridge *et al.* 1980, but symplastic transport in 'vessel' hyphae might be possible as well. Water must move in the symplast also in the case of individual extramatrical hyphae taking up water from soil, which, in analogue to root hairs, are not necessarily of higher conductance to water than soil (Weatherley 1982). Therefore the pathway of water across the cortex could be more arduous in mycorrhizas: apart from the structures of the host cells, the water would have to pass through the fungal membrane, as there is no symplastic connection between fungal cells and host root cells (Harley & Smith 1983). This pathway would be of lower conductance whether it was compared to water moving in the apoplast or symplast of the root cortex. Weatherley (1982) calculated that water moving in the vacuolar pathway, or crossing the membrane on entering and leaving each cell, would encounter a drop of water potential of 0.1 to 1.0 MPa per membrane. As the water potential of mycorrhizal plants was about 0.1

MPa lower than that of nonmycorrhizal in Experiment 3a, it is possible that this difference was caused by the water crossing more membranes in the mycorrhizal roots. This concept is also supported by the finding of Duddridge *et al.* (1980) that radioactivity from tritiated water fed to mycelial strands accumulated in the sheath region, suggesting an increase in resistance compared to strands.

Decreased root conductance may be due to the relative thickness of mycorrhizal roots as well; Sands *et al.* (1982) reasoned that, assuming the endodermis to be the site of the major resistance, mycorrhizal infection should decrease the hydraulic conductance per unit root surface area, as the surface area at the soil interface is increased more than the area of the endodermis. As mycorrhizal structure generally increases the absorbing surface of the root system in conditions other than experiments in small pots, it would be logical to suppose that the amount of water taken up per unit of root per unit time would be reduced, as this would prevent gradients of soil water content from occurring around individual absorbing surfaces (Reid 1979).

The latter argument highlights the difficulty of extrapolating from experiments in unnatural conditions involving limited rooting space and access to soluble nutrients. Therefore the value of the result on ectomycorrhizas decreasing water uptake is not so much in direct applicability to natural situations, but rather in demonstrating that mycorrhizal benefits regarding plant water relations will probably be found to be caused by the known mycorrhizal effects on nutrition and root and mycelial extension, as well as the longevity of functional mycorrhizas. Similarly, experiments in hydroponic and semihydroponic systems comparing nutrient uptake and carbon assimilation of mycorrhizal and nonmycorrhizal plants are important in increasing our understanding of the fundamentals of mycorrhizal habit, by demonstrating what ectomycorrhizas are *not* doing in natural environments - they do not appear to increase photosynthetic rates enough to increase growth rates unless nutrition is a limiting factor (Nylund & Wallander 1989), they do not necessarily increase the uptake of soluble nutrients unless rooting density is a limiting factor (Alexander & Fairley 1986, Ingestad *et al.* 1986), they do not increase plant conductance to water - thereby this work helps us to subsequently focus attention on what the mycorrhizal role really is. From the point of view of applied science, it would be appropriate to study mycorrhizal effects on drought resistance by taking into account the nutrient and root and mycelial

extension effects of mycorrhizas rather than continuing with the approach used in Experiment 3, which does not reflect real situations. In particular, quantifying the effects on water relations of variation in rooting volume, rooting density, and amount of extraradical mycelium could be rewarding.

Nevertheless, eliminating mycorrhizal effects on nutrition and the effective absorbing surface should be useful in examining some fundamental questions which as yet have not received much attention. These include confirming the evidence accumulated so far on lower conductance to water in ectomycorrhizal plants, as well as the location of the lowest conductance, as there is not much direct evidence of lowered root conductance, apart from the study by Coleman *et al.* (1987). As many aspects concerning the pathway of water across the cortex and root conductance to water are still poorly understood (Weatherley 1982), it is conceivable that study of mycorrhizal and nonmycorrhizal root systems could contribute towards our general knowledge of water uptake. Ectomycorrhizal and nonmycorrhizal (or VA mycorrhizal) roots provide a natural comparison between two possibly different pathways for water transport, which could yield insights into the uptake process.

Whether ectomycorrhizas influence the components of water potential or osmotic adjustment in shoots or roots, is still unresolved despite many hypotheses involving this effect. This problem was not tackled in this thesis, but the experiment on tissue water relations of differentially fed plants can be regarded as a first attempt for an alternative consideration of mycorrhizal effects: if it can be demonstrated that the mycorrhizal effect is mainly a nutrient effect in some circumstances (in particular, when rooting volume and density are not factors affecting water uptake), it should be possible to obtain indirect information on mycorrhizal effects by studying nutrient effects. This is relevant here in the case of tissue water relations, as there is little information of nutrient effects on them in conifers. It is time to give up uncontrolled experimentation on the physiology of different mycorrhizal plants, if inoculation proves to be only an alternative way of producing plants with different nutrition and size.

Another question which seems worth investigating on comparable mycorrhizal and nonmycorrhizal plants, is the possibility of direct hormonal control of stomata by a 'message' from roots, overriding effects of leaf water status or atmospheric influences on stomata. On the basis of experiments reported in

this thesis, this can be only speculated upon. However, as more and more information is accumulating on such phenomena (see Section 2.1.1.), and as it is the mycelial sheath and extraradical mycelium in ectomycorrhizal plants which are in contact with drying soil, rather than roots, this effect could be expected to be different in mycorrhizal and nonmycorrhizal plants, and probably different in ecto- and VA mycorrhizal plants as well. This problem could be investigated by split-root experiments, or in divided root chambers to study possible mediation of information from strands to stomata.

9.2.2 Growth and nutrient uptake during drought

The second hypothesis propounded was that mycorrhizas increase drought resistance during repeated drying and rewatering by enhancing root growth and nutrient uptake, and that this effect is related to the ability of the fungal species to form mycorrhizas during water stress. Implicit in this hypothesis is that drought decreases root growth and nutrient uptake at least in nonmycorrhizal plants, and mycorrhizal colonization at least by some fungi. The assumption of decreased root growth was found to be true in most experiments, especially in terms of root tip numbers; root dry weight was less sensitive than root tip initiation or shoot growth. However, this part of the hypothesis was not unconditionally supported by the data, since little difference was found in the growth and growth allocation between nonmycorrhizal plants and plants mycorrhizal with *Paxillus involutus* during drought, when they were adequately fertilized (Experiment 5, Chapter 7). Nevertheless, considerable differences between the isolates of fungal species used were found in this respect, and there was some indication of improved performance during cyclic drying and rewetting brought about by infection by *Thelephora terrestris* compared to other fungi, or nonmycorrhizal roots. Plants inoculated with this fungus maintained their root tip numbers at the same level as watered controls, unlike other inoculation treatments in Experiment 5, in which the drought treatment was imposed after mycorrhizal colonization was already extensive, although this effect was not found when drought began at the time of inoculation (Experiment 6, Chapter 8). Also, *Thelephora terrestris* mycorrhizal plants had somewhat less increased root / shoot ratios in dry conditions relative to other inoculation treatments in both experiments.

The relationship between the efficient colonization of root systems by

Thelephora terrestris in dry conditions and its widespread occurrence in diverse habitats has already been discussed in Chapter 8. However, the reasons for the relative efficacy of this fungus remain obscure. One possibility is that it is particularly efficient in altering the root exudation pattern and thereby extracting carbohydrate to support fungal growth in various environments. If the amount used for extensive fungal growth is small compared to root biomass increment, this would explain the somewhat smaller root / shoot ratios in these plants.

Another effect implied in the hypothesis concerned reduced nutrient uptake from dry soil, which is a well-documented phenomenon (Section 2.2.2.). However, in this work a reduction in nutrient uptake during drought was only found in one experiment, Experiment 1 before rewatering, but in all other experiments droughting either did not affect N, P and K concentrations in shoots, or increased them concomitantly with a decrease in shoot growth. As discussed before, the plants were probably able to take up nutrients rapidly during a relatively short period of available water and nutrients after rewatering. The ability of plants to exploit such short mineral nutrient pulses may confer selective advantage in habitats with low nutrient availability, and this may be more important than ability to take up nutrients from continuously low concentrations (Campbell & Grime 1989). In field conditions this effect may be more important than in these experiments, in which the normal soil microflora was not present, as transient nutrient pulses may occur in soil as a result of death of microorganisms during drought, and thus released nutrients may be rapidly reabsorbed unless they are taken up by plants (Campbell & Grime 1989).

Hence the survival by mycorrhizas of drought and sustained mycorrhizal formation by fungi such as *Thelephora terrestris* and *Cenococcum geophilum* (Pigott 1982) might be important in terms of nutrient absorption rather than water absorption in conditions where the topsoil frequently becomes dry and is rewet by light rains. Confirming this effect requires further study on plant nutrition during cyclic droughting and rewatering, involving several species of fungi. Moreover, in some climates, situations may arise in which the topsoil becomes dry enough to allow reverse flow of water from mycorrhizas to the soil, whilst deeper roots can still provide enough water to prevent dehydration. Hence water would be transported to the topsoil, which would increase nutrient availability in the layers where most mycorrhizas and highest nutrient

concentrations are. The occurrence of reverse flow is still putative, but an advantage of it would be allowing the uptake of nutrients which would otherwise be inaccessible (Passioura 1988).

In a discussion on the distribution of ectomycorrhizas with different types of mycelium, Read (1984) suggested that the abundant extraradical mycelium found in boreal and temperate coniferous forests may be an adaptation to chronically low availability of nutrients, particularly nitrogen, whereas the smooth mycorrhizas with thick sheaths typical of beech forests are more adapted to infrequent short periods of available nutrients, which can be taken up rapidly and stored in the sheath. If one of the important roles of extraradical mycelium was uptake of large quantities of water, it would be difficult to explain, why more of this mycelium occurs in northern forests, which are less susceptible to serious summer drought than those in the southern part of the northern temperate zone. In view of this, and the results discussed in the previous section, it may be suggested that the water uptake function of the extraradical mycelium is ancillary to nutrient uptake in forests.

9.2.3 Conclusion

In conclusion, mycorrhizal structure appears to improve the performance of Sitka spruce seedlings during dry conditions, but this is due to increased absorbing surface, and increased nutrient uptake from soil which results in increased water uptake. If there are plenty of soluble nutrients available, differences between fungal species may be larger than differences between mycorrhizal and nonmycorrhizal roots, however, this does not reflect natural conditions. Sustained mycorrhizal formation and stimulation of root tip initiation during cyclic drying and rewetting of soil are features of some fungi but not all, and may confer greater drought resistance to plants infected with these fungi particularly by means of improved nutrition.

9.3 Silvicultural implications

As stated at the onset of this work, the possible practical applications of results on mycorrhizal water relations are in the outplanting stage of nursery-grown containerized seedlings and bare-rooted transplants. Here, as

before, employing different time scales to consider the mycorrhizal effects can be useful.

Water deficits are probably the most important stress factor during the first days or weeks after outplanting, before the seedling has established contact with the surrounding soil, and at this stage, nutrient uptake is of secondary importance as the mineral nutrient reserves of planting stock are normally adequate (Burdett *et al.* 1984). Even though the present work did not yield evidence of better performance of mycorrhizal plants than nonmycorrhizal in response to drought in undisturbed soil conditions, the difference to outplanting situations is that planted seedlings are subjected to mechanical damage and possibly root exposure, which may be less harmful for mycorrhizal roots. In establishing contact with the surrounding soil, the mycorrhizal hyphae have been found to be more rapid than Sitka spruce roots (Coutts 1982a). Work in this thesis indicated that differences between fungal species may be important in this respect, as the fungi - particularly *Thelephora terrestris* in these experiments - which formed mycorrhizas and stimulated root tip initiation in droughted experimental conditions, can be expected to be able to grow in dry soil in outplanting sites as well.

Later in the season, nutrition may become a growth limiting factor in some sites (Burdett *et al.* 1984), and again, differences between fungal species are important in this respect; in these experiments, *Thelephora terrestris* was better able to take up nutrients from dry soil than the other fungi, even though this effect was probably rather due to its efficient mycorrhizal formation and effect on root tip initiation than improved nutrient uptake per mycorrhiza. In the longer term, competition with native soil fungi must be taken into account; if there are mycorrhizal fungi present, which are better adjusted to the particular conditions in the site, they are likely to replace any inoculated or naturally occurring nursery fungi, and hence render the inoculation procedure questionable. This is another reason why the selection of fungal species to be used for inoculation must be done with regard to the conditions of the outplanting site. One or even a few superior strains cannot be expected to improve seedling performance in all possible sites.

Apart from drought, outplanted seedlings may be exposed to other environmental stresses such as temporary flooding and extreme temperatures, and the interactions of these may result in more serious stress than any one

factor. In particular, seedlings planted in the spring may be subjected to drought whilst the soil temperatures are low, and water stress may be exacerbated by the effects of temperature on water viscosity, root permeability, and root growth (Kaufmann 1975, Lopushinsky & Kaufmann 1984, Rikala & Puttonen 1988, Grossnickle 1988a). Therefore, if mycorrhizal fungi are to be tested in relation to their resistance to environmental stresses, the stress factors must be carefully selected. Nevertheless, testing fungi in pot experiments in controlled stress conditions seems to be a valid tool for selection of fungal species and strains for inoculation programmes.

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APPENDIX A

Tables 4.1.a–4.4.a showing means and analysis of variance of original data from Experiment 1a, before discarding replicate plants with contamination.

Table 4.1.a Numbers of root tips and mycorrhizal percentage of Sitka spruce seedlings either inoculated with *Paxillus involutus* (ECM) or noninoculated (NM), ± s.e. Harvest 1: 9 weeks after inoculation (n=32), harvest 2: 12 weeks after inoculation (n=16), w = well-watered, d = not watered for 3 weeks, harvest 3: after 1 week's recovery (n=16). * indicates significance at 0.05 level, ** at 0.01 level in analysis of variance.

Harvest 1

	Number of root tips	*Paxillus* mycorrhizas %	Other mycorrhizas %
ECM	173±10	18±3.8	3.9±0.3
NM	121± 6	0±0.0	0.2±0.2
Significance of differences of means			
ECM-NM	**	**	ns

Harvest 2

	Number of root tips	*Paxillus* mycorrhizas %	Other mycorrhizas %
ECM w	375±34	34±6.0	1.4±0.9
ECM d	247±25	46±8.2	0.0±0.0
NM w	199±29	0±0.0	5.1±2.2
NM d	164±12	0±0.0	0.0±0.0
Significance of differences of means and interaction (i.a.)			
ECM-NM	**	**	ns
w - d	**	ns	**
i.a.	ns	ns	ns

Harvest 3

	Number of root tips	*Paxillus* mycorrhizas %	Other mycorrhizas %
ECM w	445±29	23±6.0	12.2±4.8
ECM d	479±61	37±7.1	9.9±6.1
NM w	277±23	0±0.0	12.2±5.7
NM d	250±21	0±0.0	27.4±6.7
Significance of differences of means and interaction (i.a.)			
ECM-NM	**	**	ns
w - d	ns	ns	ns
i.a.	ns	ns	ns

Table 4.2.a Characteristics of *Paxillus involutus* inoculated (ECM) and noninoculated (NM) Sitka spruce seedlings before (harvest 1), n=32, during (harvest 2), n=16, and after (harvest 3) exposure to drought, n=16, ± s.e. w = well-watered, d = droughted. * indicates significance at 0.05 level, ** at 0.01 level in analysis of variance.

Harvest 1

	Leaf area cm^2	Root / shoot ratio	Mean of 3 longest roots mm	No. tips / mg dwt
ECM	3.0±0.20	0.78±0.03	148± 6	6.4±0.31
NM	1.7±0.05	1.00±0.04	124±22	5.2±0.18
Significance of differences of means				
ECM–NM	**	**	**	**

Harvest 2

	Leaf area cm^2	Root / shoot ratio	Mean of 3 longest roots mm	No. tips / mg dwt
ECM w	6.2±0.31	0.70±0.04	176±8	7.0±0.53
ECM d	5.4±0.46	0.85±0.05	176±6	4.6±0.43
NM w	2.1±0.12	1.29±0.15	125±4	5.1±0.53
NM d	1.9±0.10	1.31±0.07	135±5	4.2±0.27
Significance of differences of means and interaction (i.a.)				
ECM–NM	**	**	**	*
w – d	ns	ns	ns	**
i.a.	ns	ns	ns	ns

Harvest 3

	Leaf area cm^2	Root / shoot ratio	Mean of 3 longest roots mm	No. tips / mg dwt
ECM w	6.5±0.51	0.89±0.05	193±6	6.4±0.32
ECM d	5.8±0.39	1.05±0.07	191±4	6.1±0.46
NM w	2.7±0.21	1.11±0.03	140±7	6.3±0.36
NM d	2.3±0.13	1.48±0.08	144±5	4.8±0.31
Significance of differences of means and interaction (i.a.)				
ECM–NM	**	**	**	ns
w – d	ns	**	ns	*
i.a.	ns	ns	ns	ns

Table 4.3.a N, P and K concentrations of shoots of *Paxillus involutus* inoculated (ECM) and noninoculated (NM), Sitka spruce seedlings before (harvest 1), n=32, during (harvest 2), n=16, and after (harvest 3), n=16, exposure to drought ± s.e.. w = well-watered, d = droughted. * indicates significance at 0.05 level, ** at 0.01 level in analysis of variance. If interaction of mycorrhizal and watering treatments is significant, differences between treatment means are indicated with letters.

Harvest 1

	N %	P %	K %
ECM	1.89±0.067	0.352±0.011	1.57±0.085
NM	1.80±0.059	0.077±0.011	1.17±0.044
Significance of differences of means			
ECM-NM	ns	**	**

Harvest 2

	N %	P %	K %
ECM w	1.08±0.070	0.249±0.016	1.24±0.065
ECM d	1.12±0.075	0.215±0.009	1.14±0.066
NM w	1.62±0.064	0.061±0.007	0.95±0.042
NM d	1.33±0.040	0.050±0.008	0.82±0.067
Significance of differences of means and interaction (i.a.)			
ECM-NM	**	**	**
w - d	ns	*	ns
i.a.	*	ns	ns

Harvest 3

	N %	P %	K %
ECM w	1.05±0.065	0.231±0.029	1.31±0.087
ECM d	1.17±0.078	0.270±0.021	1.16±0.058
NM w	1.43±0.069	0.042±0.008	1.14±0.062
NM d	1.59±0.050	0.074±0.009	0.85±0.045
Significance of differences of means and interaction (i.a.)			
ECM-NM	**	**	**
w - d	*	ns	**
i.a.	ns	ns	ns

Table 4.4.a N, P and K contents of shoots of *Paxillus involutus* inoculated (ECM) and noninoculated (NM) Sitka spruce seedlings before (harvest 1), n=32, during (harvest 2), n=16, and after (harvest 3), n=16, exposure to drought ± s.e.. w = well-watered, d = droughted. * indicates significance at 0.05 level, ** at 0.01 level in analysis of variance. If interaction of mycorrhizal and watering treatments is significant, differences between treatment means are indicated with letters.

Harvest 1

	N mg	P mg	K mg
ECM	0.67±0.032	0.128±0.023	0.59±0.043
NM	0.42±0.018	0.018±0.002	0.28±0.011
Significance of differences of means			
ECM–NM	**	**	**

Harvest 2

	N mg	P mg	K mg
ECM w	0.82±0.043	0.188±0.008	0.97±0.067
ECM d	0.71±0.047	0.139±0.008	0.75±0.071
NM w	0.53±0.028	0.021±0.003	0.32±0.021
NM d	0.40±0.019	0.015±0.002	0.24±0.020
Significance of differences of means and interaction (i.a.)			
ECM–NM	**	**	**
w – d	**	**	**
i.a.	ns	**	ns

Harvest 3

	N mg	P mg	K mg
ECM w	0.87±0.051	0.188±0.020	1.09±0.072
ECM d	0.85±0.040	0.200±0.015	0.88±0.071
NM w	0.57±0.039	0.019±0.004	0.47±0.042
NM d	0.58±0.033	0.028±0.004	0.32±0.030
Significance of differences of means and interaction (i.a.)			
ECM–NM	**	**	**
w – d	ns	ns	**
i.a.	ns	ns	ns

APPENDIX B

Results of Experiment 2, in which Sitka spruce seedlings were produced in 'Ray Leach' pots in perlite, in a glasshouse. Otherwise the plants were treated as described for Experiment 3, except that instead of nutrient solution with 50 mg N l^{-1}, four different strengths were used: 20, 40, 60 and 70 mg N l^{-1} solution 5 days a week, with 2 days distilled water. Half of the plants were inoculated with *Paxillus involutus,* half noninoculated. Number of replicate plants was 15. Dry weights and N, P and K concentrations of shoots were analyzed, and the results are presented in Table B.

As the nutrient regime producing inoculated and noninoculated plants with similar shoot size and nutrient concentrations was 60 mg N l^{-1}, corresponding approximately to daily watering with 50 mg N l^{-1}, this was chosen for use in Experiment 3.

Table B. Shoot dry weights (Dwt) and N, P and K % in Exp.2. Means of 15 replicates ± s.e. Differences between inoculation treatments nonsignificant. * indicates significance of differences between fertilizer treatments at 0.05 level, ** at 0.01 level. If interaction of mycorrhizal and nutrient treatments is significant, differences between treatment means are indicated with letters (Tukey's test at $p<0.05$).

	Dwt mg	N %	P %	K %
ECM				
20	99±3.7 a	2.22±.061	.271±.011	1.33±.029
40	97±6.6ab	2.57±.085	.327±.010	1.62±.034
60	90±6.9ab	2.62±.079	.358±.015	1.73±.043
70	85±5.5ab	2.78±.052	.357±.010	1.78±.032
NM				
20	74±6.6 b	2.28±.086	.289±.013	1.36±.036
40	92±6.3ab	2.35±.052	.307±.009	1.58±.034
60	91±7.7ab	2.60±.097	.369±.009	1.68±.055
70	95±5.2ab	2.64±.076	.364±.016	1.73±.040
Significance of differences between fertilizer levels				
20–40		**	**	**
20–60		**	**	**
20–70		**	**	**
40–60		*	**	**
40–70		**	**	**
60–70		ns	ns	ns

APPENDIX C

List of abbreviations used and their units if any

General

ECM = ectomycorrhiza, ectomycorrhizal

MMN = modified Melin-Norkrans medium

NM = nonmycorrhizal

VA mycorrhiza(l) = vesicular-arbuscular mycorrhiza(l)

Greek alphabet

ε = bulk modulus of elasticity MPa

ε_{max} = maximum bulk modulus of elasticity MPa

ψ_p = turgor pressure MPa

ψ_s = osmotic pressure MPa

ψ_w = plant water potential MPa

Latin alphabet

A = net assimilation rate per unit leaf area $\mu mol\ m^{-2}s^{-1}$

a = leaf area cm^2, m^2

B = apoplastic water content

c_a = CO_2 concentration in reference airstream $\mu l\ l^{-1}$

dc = difference between CO_2 concentrations in sample and reference air streams $\mu l\ l^{-1}$

dp_1 = dew point downstream of assimilation chamber °C

dp_a = dew point of background air stream °C

dwt = dry weight mg

E = transpiration rate mmol $m^{-2}s^{-1}$

e_1 = water vapour pressure in the air emerging from assimilation chamber kPa

e_a = vapour pressure of background air stream kPa

e_n = saturated vapour pressure at leaf surface temperature kPa

F = relative symplastic water content

f = mole flow of air per unit leaf area corrected to standard temperature and pressure mol $m^{-2}s^{-1}$

g_s = stomatal conductance to water vapour mol $m^{-2}s^{-1}$

J = air flow rate ml min^{-1}

L_p = combined plant and soil conductance to water

p = atmospheric pressure kPa

R^* = relative water content

r_b = boundary layer resistance m^2s^{-1} mol^{-1}

RGR = relative height growth rate mm m^{-1} day^{-1}

t_n = needle surface temperature °C

t_r = temperature of 'Rotameter' flow meters °C

twt = turgid weight mg

v_e = weight of expressed sap